THE COLLECTING NET

Vol. XVI, No. 1 SATURDAY, JUNE 28, 1941 Annual Subscription, $2.00 Single Copies, 30 Cents.

THE SPRING CRUISES OF THE *ATLANTIS* TO GEORGES BANK

DEAN F. BUMPUS

Woods Hole Oceanographic Institution

The *Atlantis* made four cruises to Georges Bank this spring, one in March, one in April, and two in May, in order to continue the studies of the productivity of Georges Bank, that very productive shoal water region extending 180 miles to the east of Cape Cod. This series of cruises during the spring months when productive rate of plants and animals is very great is a continuation of a program commenced in the spring of 1940 under the direction of Dr. George L. Clarke, of Harvard University, aided by Dr. Gordon Riley of the Bingham Oceanographic Foundation of Yale University, Dr. Mary Sears of Wellesley College, Mr. George C. Whiteley, Jr., of the Hill School, and Mr. Dean F. Bumpus of the Woods Hole Oceanographic Institution.

As in 1940 a network of about 34 stations was made during each cruise over the bank in order to measure the tem- (*Continued on page 9*)

M. B. L. Calender

THURSDAY, July 3, 1941

8:00 P. M.

M. B. L. Auditorium

Lecture:

"Current approaches to the Plant Hormone Problem."

Dr. George S. Avery, Jr., Professor of Botany, Connecticut College for Women, New London, Connecticut

The first weekly seminar of the season will be held on Tuesday, July 8.

THE PHYSIOLOGY COURSE AT THE M. B. L. IN 1941

Report of the Staff, Physiology Course

The Physiology Course is centering its work around topics in cellular and comparative physiology of marine organisms. Two two-week periods of laboratory are planned. The first of these is already proceeding under the direction of Drs. K. C. Fisher, C. L. Prosser and F. J. M. Sichel. They have designed their individual or group student research along the lines of Cellular Respiration, Comparative Physiology of Nervous Systems and the Physiology of Muscle and Peripheral Nerve, respectively.

The second two-week period of the course will be devoted to studies in Cytochemistry; Water Balance in Marine Organisms; Properties of Cell Membranes and Blood Gas Studies. This will be conducted by the new members of the staff, Drs. R. Ballentine, R. T. Kempton and A. K. Parpart.

In the above program the wealth of marine material available is being exploited to its fullest

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Photograph by Howard M. Wood, New Bedford, Mass.

AN AERIAL VIEW SHOWING THE LOCATION OF THE THREE BIOLOGICAL LABORATORIES IN WOODS HOLE

extent. It is hoped that by a well-rounded laboratory plan the interest and enthusiasm of the student will be kept at a high pitch. The laboratory work is supplemented by daily lectures by the staff and as many as possible of the research specialists working at the laboratory.

Following the first four weeks, the final period of the course will entail individual work by the students on a problem of his or her choice under the guidance of a member of the staff.

RELATION OF MACRONUCLEAR REGENERATION IN *PARAMECIUM AURELIA* TO MACRONUCLEAR STRUCTURE, AMITOSIS AND GENETIC DETERMINATION

Dr. T. M. Sonneborn
Department of Zoology, Indiana University

In recent years the study of unusual types of nuclei has been remarkably fruitful in the field of cytogenetics. The present paper is the first step in a study of a long known but still almost completely enigmatic type of nucleus.

The nuclei in *Paramecium* and other ciliate Protozoa appear in two forms, as micronuclei and macronuclei. Every normal individual has at least one nucleus of each of these two kinds. The micronuclei are in all important respects typical nuclei with chromosomes that behave as do chromosomes in higher organisms. But the macronucleus, though it develops from a micronucleus, shows no chromosomes and no indication of the precise mitotic and meiotic nuclear behavior typical of other nuclei at the time of cell division and gamete formation. Moreover, at times of fertilization it unravels into a complex skein which breaks up into many pieces; and the latter are absorbed in the cell. The lost macronuclei are then replaced by new ones formed from products of the micronucleus. In spite of its peculiar organization and behavior, the macronucleus is clearly essential for the life of the cell, while the micronucleus, though a typical nucleus, can be dispensed with. Of the many perplexing questions raised by such a strange situation, the present paper deals primarily with the following: (1) How does it happen that the macronucleus appears to divide directly without going through the complex processes of mitosis? (2) How do the micronuclei and macronuclei share and interact in the determination of hereditary characters? As will appear, the answers to these questions, in so far as they can now be given, depend upon the discovery here reported of a new and unique method of nuclear reorganization.

Paramecium aurelia undergoes fertilization during the processes of conjugation and autogamy. For present purposes the only important aspect of these processes is the behavior of the macronucleus already mentioned: its unravelling into skeins, fragmentation of the skein into pieces, absorption of the pieces, and development of a new macronucleus from the micronucleus. I have found that, at times of such reorganizations, occasionally the micronuclei fail to produce new macronuclei. This of course is inevitable when micronuclei are absent, as they sometimes are; but it also happens sometimes even when micronuclei are present. In the latter case, there is some evidence to indicate that the micronuclei did not fuse in fertilization, but remained and multiplied in the reduced condition. In either case, the cell commonly solves the problem of getting a new macronucleus, so essential for its life, by an alteration in the behavior of the many fragments of the old macronucleus. Instead of becoming absorbed in the cell, they grow up into new macronuclei. Thus, as many as 35 or more new macronuclei may arise from the 35 or more fragments of the old disintegrated macronucleus. These are segregated out to the various cells formed at successive fissions, until only one remains per cell and they then begin to divide at fissions like regular macronuclei. This behavior is repeated at subsequent reorganizations: the regenerated macronucleus or its descendant unravels into a skein and breaks up into fragments which again regenerate new macronuclei. By inducing another reorganization early in the process of macronuclear regeneration, before the fragments have reached full growth and, indeed, before their number has been reduced to one per cell, one can observe that the disintegration of these incompletely developed macronuclei varies with their degree of development: when quite small, they form no skein at all; when larger, they form a very simple skein; when still larger the skein becomes more and more complex until at full growth it closely resembles the skein of normal macronuclei. For comparison, one may observe the behavior of still unabsorbed macronuclear fragments in individuals that have in addition a

The Collecting Net was entered as second-class matter July 11, 1935, at the Post Office at Woods Hole, Mass., under the Act of March 3, 1879, and was re-entered on July 23, 1938. It is devoted to the scientific work at marine biological laboratories. It is published weekly for ten weeks between July 1 and September 15 from Woods Hole, and is printed at The Darwin Press, New Bedford, Mass. Its editorial offices are situated in Woods Hole, Mass. Single copies, 30c by mail; subscription, $2.00.

macronucleus formed in the normal way. When such animals are induced to conjugate, the old fragments never form skeins, but behave just as do the regenerating fragments before much growth has occurred. These observations demonstrate therefore, that the complexity of the macronuclear disintegration skein reflects a corresponding complexity in the organization of the macronucleus; and, what is more important, they prove that the macronucleus is a compound structure with each of its component parts (corresponding to the more than 35 fragments it forms) containing all that is required for the development of a complete and functional macronucleus, that is, a complete genome.

This is the clue to the answer of our first question. The macronucleus appears to divide amitotically because it is a compound nucleus, not a simple one; it contains more than 35 sub-nuclei and at division all that is required to yield genetically equivalent functional macronuclei is to segregate these multiple subnuclei into two groups. Of course multiplication of the subnuclear components must take place at some stage of the nuclear cycle and this must now be sought for, not in the behavior of the nucleus as a whole, but in the behavior of the parts within it. Perhaps it occurs during the profound and little understood changes known to precede the gross amitotic division. Further study should now be directed on this point.

Animals in which macronuclear regeneration has taken place often die or produce some nonviable progeny; but many live and produce a line of descent in which all subsequent reorganizations are invariably by the same method. Such cultures are always of the same mating type as the original parent cell, thus further supporting the view I have previously maintained, that the macronucleus directly determines mating type. On the other hand, a whole complex of new characters invariably appears in these cultures: reduced size, fission rate and viability; very short interreorganization periods; and extremely intense sex reactivity. The direct cause of these characters is not fully known, but it is of interest that the size and viability appear to be reduced more when micronuclei are lacking than when they are present, thus implying a direct effect of the micronuclei on these characters. Further, the macronuclei in such animals are also reduced in size, suggesting a direct correlation between macronuclear size and cell size, and an inability of the single macronuclear fragment to grow into a macronucleus of full size. The latter fact may also play a part in determining the low vigor of such animals, their strong mating reactivity and their short interreorganizational periods. Regenerated macronuclei, though adequate to support life and reproduction, may still lack something required for perfectly normal functioning.

The results here set forth show (1) that the macronucleus is compound, (2) that the skein into which it disintegrates is a reflection of the complexity of its organization, (3) that macronuclei regenerated from fragments are nearly, but not quite, perfect and so determine corresponding imperfections in the cell, and (4) that micronuclei, though not indispensible, nevertheless have certain direct effects on cell characters. These are first steps towards a solution of problems of cytogenetics in *Paramecium aurelia*; further steps in the same direction should follow from the opportunities here provided for studying separately and in various combinations the physiological and genetic roles played by micronucleus, normal macronuclei and imperfect regenerated macronuclei, and from the methods here reported for attacking problems of macronuclear organization.

(Summary prepared for the press of a paper presented before the American Society of Zoologists at the meeting of the American Association for the Advancement of Science on January 1, 1941.)

INFORMAL NOTES ON *EULIMA* EMBRYOLOGY BY THE EMERITUS CURATOR OF THE M. B. L. MUSEUM

George M. Gray

[**Note:** Mr. Gray has been associated with the Marine Biological Laboratory for fifty years as collector, curator of the Supply Department and lastly as Museum curator.]

PREFACE

In August 1932 the author published in The Collecting Net an article on the habits and habitat of this dainty little mollusc. There was nothing whatever in the article about its breeding however. The following embryology comprises just a few notes taken during the last two years.

Eulima olecacea, of Kurtz and Stimpson, or *Melanella oleacea* of other authors, is a beautiful small gastropod mollusc of a creamy-white color, with jet black eyes. The shell is finely polished. It tapers to a fine point, and the largest one I have is barely 10 mm. long or 6/16 of an inch, about 3 mm., 1/8 of an inch, at broadest part with 12 whorls, finely and delicately separated by lines. It is certainly a dude among our small molluscs. It can sometimes be taken by dredging but as it is a "quasi parasite or commensal", on the black sea cucumber (*Thyone briareus*), it is easier to get them by collecting the sea cucum-

bers, though one time I handled something like 250 *Thyone* and got seven *Eulima,* and two of these were on one sea cucumber. I have had better luck at other times.

Recent Observations

July 14, 1939. On the morning of July 8th just before noon, one of our collectors brought me among other small molluscs, ten *Eulima oleacea,* alive and some of them attached to *Thyone briareus.* I took the snails and put them in a separate finger-bowl of sea water and let them stand.

The next morning there were in the bowl several small white specks or spots, somewhat smaller than the head of an ordinary pin. They did not seem to mean much, but on investigation proved to be the egg capsules of the *Eulima.* There were evidently a number of individuals packed closely together and surrounded by a sort of jelly-like substance—something like that which surrounds the eggs of the frog. There were only a few of these capsules at first.

On my next looking, which was the following morning, I found the number had increased to perhaps 18 to 20, which means that a single snail laid more than one capsule. About this time I took six of the snails and placed them in a separate finger-bowl of sea water, leaving four snails in the bowl with the eggs. I examined the eggs for two or three days. I observed no sign of life, but each individual was evidently larger and beginning to separate from the others. Though no movement could be observed with the microscope I was using, the eggs were undoubtedly in the cleavage stages. (My microscope was not high powered enough to show.) On Friday morning on looking at them, I found that each individual had separated from its companions and all were whirling around or moving about more or less rapidly. They looked as though they thought they were going places, but all this action was taking place *inside* of the surrounding jelly. These individuals were rather perplexing to count as they did not stay put. We judged that each capsule contained from fifty to sixty separate individuals and were in what is known as the veliger, or larval, stage.

The eggs were laid on the bottom and sides of the finger-bowl below the surface of the water. Up to date (July 14) have noticed no eggs from the six snails which I had separated from the original lot. I doubt if any more eggs have been laid by any of the ten original snails. Friday afternoon of the 14th I placed a *Thyone* in the bowl which had the six *Eulima.* In about a half hour or perhaps longer, two of the snails had settled on the *Thyone,* and in less than four hours, all but one were on the same sea cucumber. The last three to go on may have gone on in less than the four hours as I was busy at other work, and did not keep too close a watch.

July 15. The next morning all six were on the *Thyone.* I then took the *Thyone* out, removing the snails, and put all the snails in a bowl of clean sea water with *no Thyone* to see if by chance their night's repast had put them a better humor for laying or if they had laid out their supply.

I washed the *Thyone* to see if there were any eggs laid on it, but observed none in the wash water. They would be difficult to find on the *Thyone.* In the meantime there seemed to be two clusters of fresh laid eggs. The earlier eggs were in the veliger stage, while those that seemed fresh laid were very quiescent, probably in the cleavage stage. (These two capsules of fresh eggs were in the bowl with the original eggs and not in the *Thyone* lot.)

The eggs which I first found in the veliger stage were seemingly more energetic than ever the morning of the 15th, still in the jelly but the whole capsule, or capsules were enlarged over what they were at first (or larger than they were on the 14th). The individual veligers seemed larger also. It would seem that the walls of surrounding jelly had been inflated or pushed out all around, something like a toy balloon is made larger. This of course would create a greater space for the veligers to perform their gyrations, though they still bumped elbows.

July 16—past 9 A. M. Veligers larger, still in the capsule, and still think they are going places very rapidly. This morning I discovered six egg capsules in the bowl with the six *Eulima* which I had taken away from the *Thyone* yesterday. I think one was laid yesterday before 6 P. M. These were evidently in early cleavage stages. There was no individual movement. These capsules were on the sides of the bowl. At 8:30 P. M. there were ten egg capsules in the finger-bowl, three of them on the bottom of the bowl. None of the first laying had left the jelly but had increased in size especially as individuals. Very active. On examination none of the fresh, or last laid eggs, had shown any separate individual motion or swimming, but were all adhering together.

July 17 — 8:30-9:30 A. M. None of the veligers have left the mother capsule as yet. Still very lively and seemingly larger. Does not seem as though they could get along in their cramped home much longer without serious friction. In the finger-bowl containing the six *Eulima* which had been on the *Thyone* there were twenty-one egg capsules as against the ten of last night. Of the eleven additional, most of them were on the sides of the bowl, one or two barely covered with water, one at the very surface. Some of the last laid were very fresh, evidently laid early this morning. All the capsules were still holding the

individuals closely packed. No veligers yet (10:15 A. M.).

At 6 P. M. one more capsule of eggs with the six *Eulima* still closely packed, but evidently beginning to separate more, but none free-swimming. No veligers, though one had clearly separated from the others in one of the capsules.

July 18 — 9 A. M. There were twenty-eight capsules of eggs in the finger-bowl with the above six *Eulima*. Beginning to show clearer individual outlines. Still growing, but not veligers. The *Thyone* feed was evidently an inspiration to the *Eulima*.

Twelve fresh *Eulima* were collectly yesterday on *Thyone*, separated from the *Thyone* and placed in a finger-bowl of clean sea water, but there were no eggs this A. M. None of the earliest laid eggs have left the home brooder as yet. Still in the veliger stage and going strong.

At 5:45 P. M. evidently not much change in the above eggs. No veligers have come forth from the capsules. The snails collected yesterday have not laid. Have been partially changing water daily on all eggs.

July 19 — 8 A. M. Veligers in first lot of eggs still going strong but still in the doghouse. Seems to be no indication of coming out. A few more egg capsules were in the bowl with the same six *Eulima* as mentioned yesterday. As I disposed of a half dozen yesterday, it would mean that about ten were laid last night and late P. M. yesterday. No eggs as yet from the *Eulima* collected on the 17th. This morning I put the four *Eulima* (which had been a part of the original ten) into a finger-bowl of fresh sea water, into which I had first placed a small *Thyone*. These four had been in the same bowl since first collected and in the last two or three days had been quite negligent about laying. It may be that a good feed on the *Thyone* will pep them up to laying eggs again, like some humans who flourish at others' expense. It is only about a half hour ago that I put them in with the *Thyone* and three have already attached to the *Thyone*. At 5:45 still three on the *Thyone*, one off. May have been on and gone off again, but think not. Of the twelve collected the 17th I gave away two so that I have ten left to care for. At 5:45 P. M. one egg cluster or capsule was found with these ten. At 5:45 P. M. oldest ones still in veliger stage and inside the original capsule. Of the capsules from the six *Eulima*, the individual eggs have separated more, but none free-swimming, i.e., no veligers; perhaps two or three additional capsules.

July 20 — 9:30-10 A. M. Evidently the veliger stage with some of the egg capsules mentioned last took place last night or early this A. M. as some individuals of these eggs were freely swimming, while others were just barely showing perceptible movement. (These are from the six *Eulima* which had been separated originally.) The *Eulima* themselves had possibly not laid any more eggs since last night. Of the first lot of veligers some seem dead while others are very slow in their movements. The ten snails collected the 17th had laid six capsules of eggs as I found this A. M. I took these ten *Eulima*, put them and a small *Thyone* into another finger-bowl of sea water and now await developments (10:15 A. M.).

About noon today noticed the first coming out, or beginning to come out, of the veligers from the capsule, only a few. At 5:40 P. M. there were some with quite lively veligers. Some just moving and some not, the livelier ones are older. These were from the six *Eulima* which had been separated from the first ten of the 8th, but no *Eulima* on the *Thyone* of the ten put in this A. M. (This would mean that twelve days at least from egg to coming out of the capsule.) One went on but is off tonight.

July 21 — 9 A. M. This morning I found the wall on one side of one of the oldest egg capsules was open and a number of veligers or well-advanced larvae were swimming around freely in the water. I think the veligers had pushed out this wall and were taking advantage of their escape from the old homestead and enjoying a swim, though not in forbidden waters. Four out of the ten *Eulima* were on the *Thyone*, the other six were sulking on the sides of the finger-bowl, five were under water and one barely above the surface.

July 22 — 8:30 A. M. This A. M. four of the ten last collected *Eulima* were on the *Thyone*. The other six were mostly on the sides of the bowl. But no eggs were found either on the *Thyone* or on the sides of the bowl. Water was changed and the animals left as before, and of the four *Eulima* of the first lot, no eggs were found. The *Thyone* had eviscerated. I threw out this *Thyone*, washed the inside of bowl, put in another *Thyone* with the snails, added fresh sea water and left them to their fate. To lay or not to lay— that is the burning question.

No more coming out parties, no more debutantes, no more veligers coming through the maternal wall of the egg capsule. Those which were in the veliger stage were going lively. These are the ones from the six *Eulima* which were of the first lot collected on July 8th. Those that left the capsules yesterday are about as they were last night.

July 23 — 9:30 A. M. Found no fresh eggs from any of the *Eulima*. The two lots on the *Thyone* in different bowls had no egg capsules, neither on the *Thyone* nor on the bowl. These lots were: one lot of four from the catch of July

8th, one lot of ten *Eulima* from the collecting of July 17th. The veligers from the lot of six *Eulima* are still lively and some have come out of the capsule and are swimming ecstatically about in the water outside though most of them are in the capsule.

Many of the veligers were out skylarking around in the water outside of the capsules, seemingly having a grand outing. Several, three at least, of the capsules were empty with one exception, which had only a few. The others will have to be examined under a better light than tonight, though I did not notice any new eggs.

July 24. Things seemed to be about as yesterday. Some veligers of the first lot appeared dead. Of the egg capsules in the bowl containing the six *Eulima* of the original lot they seem to be in about the same condition as yesterday, but of course a little more advanced. Some in, some out of the capsules. Discovered no eggs in the lot of four *Eulima* with *Thyone*, or in the lot of ten. Added fresh sea water and left them.

July 25. Noticed no particular change in any lot and found no fresh eggs in any lot changed as above. No eggs for several days.

July 28. The ten *Eulima* collected the 17th and which had been on the *Thyone* and then taken off and put in a finger-bowl by themselves lately had two egg capsules in with them. I discovered this A. M. some of the other veligers are still living.

August 4. So far as I could judge no new eggs of *Eulima* have been laid for several days. On July 31st six *Eulima* just collected were brought to me and put in a separate dish. On August 1st and 2nd more just collected *Eulima* were brought to me and on August 2nd one more freshly collected *Eulima* was given to me. These were all placed in the same dish with those of July 31st, and could not discover that any of them had laid.

On August 3rd two additional freshly collected *Eulima* were left in a three-ounce corked vial of sea water overnight; I found one or two egg capsules on the side of the vial. I at first thought only one but on pipetting them out was surprised to find two whole capsules. These two *Eulima* and their eggs I am keeping in a separate finger-bowl from the others.

August 6. Two egg capsules in the bowl of six *Eulima*. None from last collected *Eulima*.

August 11. I think it was on August 7th or not later than the 8th that I gave the last collected lot of *Eulima* (those of July 31st and August 1st and 2nd) a change to a bowl of sea water containing a *Thyone*. Up to this time none of this lot had laid eggs. There were very small patches or spots of jelly or mucus, but no well authenticated eggs in them.

(*Concluded Next Week*)

THE NATIONAL EDUCATION ASSOCIATION AT WOODS HOLE

At the suggestion of Mabel Studebaker, formerly a worker at the Marine Biological Laboratory, and now Northeastern Regional Director of the Classroom Department of the National Education Association, the executive board of that organization decided to hold its business sessions at Woods Hole for the two days preceding the Boston Convention.

Dr. Charles Packard of the Marine Biological Laboratory very generously extended the facilities of his institution to the group. Meetings were held in the Committee Room of the Administration Building. The ten members of the committee representing teachers from the four corners of the United States were housed in the new dormitory.

On Thursday afternoon, when the last member had arrived, the Executive Board seated around the table were:

Mrs. Mary D. Barnes, President of the Department, from Elizabeth, New Jersey.
Miss Margery Alexander, Vice-President, Charlotte, N. C.
Mrs. Eleanor F. Edmiston, Secretary, San Diego, Calif.
Miss Mabel Studebaker, Northeastern Regional Director, Erie, Pa.
Miss Katy V. Anthony, Southeastern Regional Director, Richmond, Virginia.
Harold H. Blanchard, North Central Regional Director, South Bend, Ind.
Miss Florence B. Reynolds, South Central Regional Director, Omaha, Neb.
Miss Mary E. Bond, Northwestern Regional Director, Bellingham, Wash.
Wilber W. Raisner, Southwestern Regional Director, San Francisco, Calif.
Miss Elphe K. Smith, former President and Director ex officio, Tigard, Oregon.

The Department of Classroom Teachers is the largest of the twenty-seven departments of the National Education Association. It is composed solely of class room teachers. They comprise 80% of the National Education Association's total membership, which includes over 200,000 educators. All teachers, public and private, in the United States or in its possessions are eligible to membership in the Association and to consequent membership in the Department of Classroom Teachers.

The Department works constantly for the advancement of the teaching profession. Its service to the teachers of this country in the establishment of adequate salary and retirement benefits has been inestimable. —MARY D. BARNES.

HANS DRIESCH, PHILOSOPHER AND SCIENTIST

Ralph C. Busser

American Consul General at Leipzig, Germany, until 1940, Philadelphia, Pa.

On the 16th of April, 1941, Hans Driesch, the world renowned German philosopher and scientist, died in his 74th year at Leipzig, Germany. In October, 1933, he had retired from his position as professor ordinarius at the University of Leipzig, receiving from the Saxon Ministry of Education the title of "professor emeritus." Driesch stands out as one of the greatest thinkers and educators of the world, and very few university professors have done as much in promoting intellectual cooperation between nations and a sympathetic understanding of the mentality, ideals and achievements of his own and other countries in the realm of science and culture.

Hans Driesch was born in 1867 at Kreuznach (Rhineland). His father was a wholesale merchant in Hamburg, where he got his early education. Hans Driesch, who became a doctor of law, medicine and philosophy, began his career as a zoologist and was engaged in research work at the zoological station in Naples from 1891 to 1900. Later he devoted his studies to philosophy, teaching it from the viewpoint of natural science rather than as a dogmatic metaphysician. His most famous work is "The Science and Philosophy of Organism" (1907/8), which was first published in Great Britain, as it consisted of the "Gifford Lectures" for which he received the Gifford Prize at the University of Aberdeen, where he taught philosophy from 1907 to 1908.

In 1909 Driesch became a Privatdozent (lecturer) at the University of Heidelberg, where in 1911 he was appointed professor extraordinarius. In 1912 he published his second principal work called "Ornungslehre" (Logic), then in 1917 the "Wirklichkeitslehre" (Metaphysics), both of these works together forming a complete system of philosophy. In 1920 Driesch became professor ordinarius of philosophy at the University of Cologne.

In 1921 Driesch was appointed to the same position at the University of Leipzig, but in 1923 he was abroad for more than a year giving lectures in China, Japan and the United States. While in China, Driesch was guest professor at the Chinese State University in Nanking and Peking, where he succeeded Professor John Dewey, the famous American writer on philosophic and political subjects.

In recent years Professor Driesch has taken a great interest in the problem of psychology and wrote "Leib und Seele" (Mind and Body), and "The Crisis in Psychology" (Princeton, 1924). Driesch is also famous for his studies in psychical research. His book on "Para-psychologie" created a sensation in the scientific world.

In 1926 Driesch was granted a term's leave of abscence from the University of Leipzig, which period he spent at the University of Wisconsin in Madison, to which he was appointed "Carl Schurz Memorial Professor". He has also lectured at the English universities of London, Manchester, Leeds and Cambridge, at the national University in Buenos Ayres, in Italy, Switzerland, Czecho-Slovakia, and other countries.

Until his recent retirement Professor Driesch was Director of the Philosophical Institute at the University of Leipzig. He received the title of honorary doctor at a number of universities, was formerly president of the Society for Psychical Research in London, and up to the time of his death was a member of many other scientific and learned societies. Driesch was a fine linguist, speaking English, French and Italian fluently as well as his own mother tongue.

Driesch was one of the most popular professors at the University of Leipzig, students flocking there from all parts of the world to listen to his lectures. He was an international personage not only through his experience as a lecturer at numerous American and foreign Universities, but more through his celebrated works on philosophy and psychology, which have challenged old theories and opened up new paths of thought.

During the past ten years I had many conversations with Dr. Driesch in Leipzig, where I was stationed as American Consul General until my retirement from the Foreign Service in January, 1940. As Dr. Driesch never accepted the Nazi philosophy but clung to his lifelong belief in liberal and democratic principles, he was never permitted, after 1933, to lecture or hold public addresses in his country. The loss of academic freedom in Nazi Germany, even in non-political fields of learning, and other restrictions upon intellectual and religious activities had a stifling effect upon Dr. Driesch, whose literary work be-

came practically sterile. No philosophy except that of despair could thrive in such a prison-like atmosphere.

Meeting Dr. Driesch frequently at our respective homes and elsewhere in Leipzig, I greatly enjoyed his friendship and learned to appreciate his splendid character, broad-minded views, and intellectual and social qualities. Dr. Driesch was a popular figure in University and other circles in Leipzig, and his conversation was always sprightly and entertaining. Although one of the greatest intellectuals of his time, he never made a display of his knowledge in society, rather talking on subjects most interesting to his particular listener. Dr. and Mrs. Driesch frequently entertained at their home, which was on the floor above my home in Leipzig, and their guests were always sure of a hearty welcome and the traditional German hospitality. Their children, Kurt and Ingeborg Driesch, have already achieved considerable success in the musical world, the former as a conductor and composer, and the latter as a violinist; each of them has held many concerts in Germany during recent years.

THE SPRING CRUISES OF THE *ATLANTIS* TO GEORGES BANK

(Continued from page 1)

perature and salinity of the water at regular intervals. From these same levels samples of water were taken in order to measure the concentration of O_2, Nitrate, Phosphate and chlorophyll as an indicator of the abundance of plant pigment which in turn is an indicator of the abundance of phytoplankton. Samples of sea water were also taken from these same levels for numerical and taxonomic studies of the phytoplankton and nannoplankton species, the latter in which Dr. James B. Lackey, of the U. S. Public Health Service, is interested.

Oblique tows for zoo-plankton, copepods, schizopods and decapod larvae, other small crustacean forms, sagittae, Haddock eggs and larvae, were made with the recently developed quantitative Plankton Samplers from two or three strata depending on the depths in order to sample the whole water column. Other tows were made from the bottom to the surface with meter and a half stramin nets in order to sample the larger members of the plankton population.

It is hoped that not only will a good picture of the productivity of the region be developed, but that factors influencing the survival of young haddock be obtained. At present it is not at all clear whether biological or physical factors or both are responsible for the large loss of young haddock in this peculiar marine aquarium.

We hope soon in the future to continue the collection not only of the plankton on Georges but also a study of the fauna living on and in the first few centimeters of the bottom. Photographs made last spring by Dr. Maurice Ewing of the bottom of Georges Bank have whetted our interest and enthusiasm for a complete survey of this phase of the problem which is so important in the economy of the sea. We plan this summer to develop a clam shell dredge for just such a quantitative study.

DEPARTMENT OF CHEMICAL SUPPLIES AND SCIENTIFIC APPARATUS

Apparatus, Room 3; Chemicals, Room 8
Office, Room 1, Marine Biological Laboratory

This department loans without charge to properly qualified research workers at the Marine Biological Laboratory, chemicals and ordinary glassware in reasonable amounts. Certain types of special apparatus, required by investigators for their research are also available for short periods of time, depending on the prior allocation and advance requests for such apparatus.

Members of classes are not entitled to supplies other than those provided in their regular class work. Beginning investigators will receive supplies only on the authorization of the person under whom they are working for the season.

Expensive and fugitive supplies such as, liquid air, dry ice, and gas mixtures, are paid for by the investigator. Chemicals in large quantities and those not generally carried in stock, if expensive, are also charged.

In order to carry out the usual services, the cooperation of investigators is urged in the three following requests: That

1. Supplies and apparatus no longer in use be returned. *In emergencies these may be recalled for redistribution.*

2. Glassware, including aquaria and museum jars, be cleaned thoroughly before returning to the Chemical or the Apparatus Room. *Failure to cooperate in this regard means added costs and subsequent loss of efficiency and service.*

3. If certain supplies from the Chemical Room are to be used by an investigator during the next succeeding summer he may reserve them when arrangements can be made to do so. Supplies thus required must be packed in boxes or cartons and properly labeled. A *Kept Out Card,* obtainable from the Chemical Room for this purpose, must be properly filled in and left with these containers. All supplies not thus listed, packed, and marked, will be returned to stock.

PHYSIOLOGY CLASS NOTES

"This is a course in Physiology," quoth Dr. Parpart, as we, on the morning of June 17th, sat in the lab patiently awaiting our first day of work. After our first week of strenuous labor in said laboratory we have come to agree not only with Dr. Parpart, but also with the janitor who said, quote "—— that course in slopology;" unquote. One can easily understand the janitor's point of view when, at the end of the day, we observe the tidy lab, with the remains of starfish, frogs and dead dogfish hanging out of the waste baskets, and the rest of the day's rubbish ankle deep on the floor. It is without a doubt the janitor's paradise.

The prevailing atmosphere in our lab is, of course, one of peace and calm,—with someone quietly yelling out manometer readings, the dogfish splashing water over everyone in protest of their tank existence, the embryologists looking for things we never heard of, Bill Keezer scratching his beard, the centrifuge centrifuging and the aspirator aspirating.

If Mr. Warburg were only here he certainly would be proud of Dr. Fisher's busy students. They have run the Warburg apparatus almost to the point of exhaustion, as observed by the mighty screech it developed and the more than once broken belt. And the results!—Enough to make Mr. Warburg sit up and take notice. To quote the cellular respirationists:—"The cells respire while we perspire."

And on the other side of the lab, trying to concentrate between the ten minute readings of the Warburg enthusiasts, we find the quieter type of student daintily gaining entrance to a limulus with a can-opener, eagerly trying his surgical skill on an unsuspecting dogfish, or solemnly studying his waves on a smoked drum. These are Dr. Prosser's students.

Back in a little room all by themselves we find half of Dr. Sichel's students—all tangled up in electrical wirings and muscles. The other half are in the air-conditioned dark room, avoiding the summer heat, measuring tensions developed by frog muscles, and developing a few of their summer snapshots on the side.

This year the laboratory work has been organized into seven sections. For the first two weeks, three sections have been running parallel, and for the last two weeks, there will be four sections. A wide choice is therefore given to each student as he may select two sections for each two week period.

The staff of instruction has been changed considerably this year. With the departure of Drs. Höber, Irving, and Shannon, have come Dr. A. K. Parpart, Associate Professor of Biology, Princeton, to take charge of the course; Dr. Robert Ballentine, Procter Fellow, Princeton; and Dr. R. T. Kempton, Professor of Zoology, Vassar.

One thing it didn't take us long to learn is that to be a physiologist one must be not only a biologist, but also an electrician, a mechanic, a chemist, a physicist, and, above all, an optimist.

—*J. E. H.*

BOTANY CLASS NOTES

Skepticism, instilled by certain members, is fast becoming the outstanding characteristic of this year's botany class. Accusations of overacting imaginations are many and severe, with the result that "Are you *sure* you saw that?" and such disparaging remarks are fast becoming botany bywords. Though they began quite locally and were only sporadic, the skepticism is increasing in direct proportion to the difficulty of the algae.

Tuesday, June 17, was the first day of a course that wasted no time in going full-steam ahead into work. Prefaced in the morning by a short introduction from Dr. Taylor, not without the interpolation of some of his inimitable quips, the study of the "Morphology and Taxonomy of the Algae" started with observation of gametes and zoospores of *Chlamydomonas* feverishly dashing across the slide like army men heading for town on a day off, and those who had never before met *Chlamydomonas* can now recognize that primitive algal cell form with its typical cup-shaped chloroplast, red eye-spot and two flagella. It is fast being discovered that a so-called "day's schedule" really includes half the night as well, and yet, Bill Gilbert, the assistant, promises that "the worst is yet to come."

Field trips, it seems, are the dessert of the course, and the first one, held last Friday, was all that the algologists had been warned it would be. So the class was eagerly anticipating the first one via boat to have been held Wednesday, the day after this report was due. However, the anticipation was slightly flavored with fervent prayers for a sunny day, after the build-up Dr. Taylor gave about the lunch question. The Mess, it seems, is quite willing to pack a lunch for its patrons, but it dislikes having excess food on hand because of rain, so that once the lunch is made,

it's made, and the multitude either eats it that day or holds it for the first decent day thereafter. If that day doesn't arrive for several moons or so, a stale Mess lunch is just another of those things one meets in a good botany course at the M.B.L. *C'est la vie, c'est la vie.*

The first field trip, already mentioned, luckily fell on a beautiful day, and the initiation was spoiled by neither rain nor cold, or even by chilly winds. Following the speedy pace set by Dr. Runk and Dr. Tseng was quite invigorating and seemed second nature to a few people, but it was soon evident that most of the class was of the rear guard variety.

The general collecting technique, one soon discovered, is to wade into the water or muck of the particular habitat, snatch up a few samples, get a "squeezing" or two, scribble a label, and then dash off on a dog-trot to catch up again with the leaders. It was when the first *Rhus toxicodendron* was encountered that the preliminaries of thoroughly covering one's exposed anatomy with the special poison ivy preventative, which gives such a lovely yellow jaundice or golden tan effect —depending on the individual's aesthetic sense— seemed less humorous and more of a pretty good insurance.

Highlight of the trip was the visit to Cedar Swamp—a real swamp, by gar, through which one carefully kept well within the limits of the knee-deep, brown-water trail, or else sank his errant foot into the depths of nothingness.

Friday being a special day for the botanists, what with the field trip and all, ended up with a bang by the first seminar meeting, at which Dr. Taylor showed some fine movies—he claims to have been an amateur when he took them, but that tale is hard to believe—and he kept up a running commentary of informative and funny remarks so that several hours were spent as none, in looking at algae, tropical flowers in gorgeous colors, and details of the Allan Hancock Exploration Trip #2 to Central and South America. (*General note*: If there's a chance for everyone to see these films, no one should miss it.) And so a wonderful time was had by all, substantially augmented afterwards by tea, Ritz cracker sandwiches, and cookies, an elaboration of the regular daily 10 p.m. custom which offers time out to late lab workers.

Of all the interesting things viewed through a lens to date, it must be admitted that one of the best was a submarine suddenly seen by Dr. Runk. Indeed, its rare appearance in the waters of Woods Hole was sufficient reason to give those who were truly scientific-minded an extra field trip to Juniper Point for closer observation. Rumor has it that a car loaded with ten people— only girls visible—caused family difficulties when viewed unexpectedly by the owner's wife. But it's these unexpected events that give the class good subject matter for dinner conversation while they're waiting for the tenth refill on the beans.

—*J. W. and C. S.*

EMBRYOLOGY CLASS NOTES

Dr. Goodrich opened the course in Embryology with a bang bright and early on the morning of June 17, 1941. After recalling to our so-called minds the history and terms with which some of us had been vaguely familiar in the past, Dr. Costello was introduced and we were plunged into the entangling alliances of sperm and egg. The Panzer divisions of the sperm being eventually victorious we moved on to cleavage and the problems of the Germinal Vesicle. Following recent work in Amphibia certain talented members of our group removed these vesicles very successfully, at first with trepidation and later with greater facility. The animal under observation was *Nereis limbata* which we observed with mixed emotions in its natural nightly activities at the dark of the moon.

The main part of the week was concerned with the highly interesting Teleost fish, species *Fundulus heteroclitus*. The struggle was long and drawn out especially when there was a traffic jam in the vitelline veins, which only Dr. Goodrich could straighten.

Dr. Schotté ended the first week with a precedent-shattering lecture for those brought up upon the theory of concrescence and was nobly aided by one battered chapeau which in the course of the lecture was caused to gastrulate, envaginate, delaminate, and enfiltrate. The chapeau recovered but the class is still considering.

Social life which started with a few hesitant dips into a cold sea and tennis by George and Neil (without a net) received a new lease on life at the mixer Saturday night. It really was a mixer! "Cutting in," an American custom at dances, was strongly objected to by some of our more distinguished members because of variances from the international norm.

The class wishes to express its gratitude to its two superior assistants—to the fatherly benevolence of Ray the success of our observations is largely due. As for Trink, it has been encouraging to see such a brilliant mind in all phases of rest and play.

—*Patricia Hollister and Elizabeth Kirkpatrick*

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris I. Gorokhoff and Judy Woodring.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879, and re-entered, July 23, 1938.

Introducing

Dr. Olga Janowitz, formerly Secretary of University Extension Courses in the University of Vienna and Lecturer in Biology in a Realgymnasium in Vienna; now Instructor of mathematics and Latin, Potomac School, Washington, D. C.

Dr. Janowitz is a native of Vienna, Austria, where she studied at the University of Vienna and received her doctorate of philosophy in both zoology and botany.

After she received her degree, she taught in a Realgymnasium in Vienna, which was attended by girls eleven to nineteen years old, and was also appointed by the school department in charge of training teachers in biology for Austrian secondary schools. While she was also interested in conducting research, she found that her inclination was for adult education. She felt that the principles of biology would be extremely useful in providing a *Weltanschauung* for the disillusioned and bewildered people of post-war Austria. In this connection she became secretary of the University Extension Courses and there she gave a series of lectures each year on biology, genetics, and heredity. Prominent in her lectures were the arguments that there was no dictatorship in nature and that there was no biological basis for racial prejudice.

Such lectures naturally brought her into conflict with the Nazis after they effected the *Anschluss* in 1938, and she left Austria a year later for England. There, under a grant from the British Federation of University Women, she conducted research with Dr. H. Hardy at the Hull University College. He had devised apparatus to collect plankton off the shores of England. This work had hardly started when it was brought to a standstill by the outbreak of war.

In March of last year she arrived in the United States, and in a few weeks she obtained a position in the Potomac School in Washington. She is interested not only in teaching, but in various other types of work. For instance, this summer, in addition to conducting research at Woods Hole, she is organizing a group to practice German conversation based on biological topics, which is meeting weekly in the Old Lecture Hall. Through this type of work she hopes to find persons who will be able to visit Europe after the war to spread democratic ideals there.

M.B.L. CLUB SPONSORS MIXER

The social season of the M. B. L. Club was opened last Saturday night with a mixer, to which everyone was invited. Miss Lucille "Sunny" Nason, the general chairman, saw to it that the long-anticipated event lived up to all expectations.

Upon entering the club, each person was given a name card to wear during the evening. The drawings of whales, plant cells, etc., to designate the various classes were done by Mary Chamberlin, Roger Cole, Arthur Woodward, and Mrs. T. H. Bullock.

Immediately catching the eye of newcomers were the unique decorations of fish nets suspended from the ceiling, holding sea urchins, horseshoe crabs, and other sea animals. The Supply Department of the Laboratory furnished the materials and those who arranged them were Dick Lee, Dr. W. W. Ballard, Dr. C. Hyman, Warren Healy, Dr. Lauren Gilman, Barbs Schraff, and Mary Donovan.

While chatting with various groups of young and old, the guests were served refreshing punch and cookies. Those in charge of the refreshments were Mrs. H. B. Goodrich, Mrs. T. H. Bullock, Mrs. Edward Warner, H. Duncan Rollason, Theodore Genther, Mrs. Karl Wilbur, Jane Henry, and Jacqueline Waldron.

Dr. E. R. Brill, Dr. K. M. Wilbur, and Mr. Richard Henry had arranged for the latest records to be played on the phonograph and before long the gathering turned into an informal dance.

As a result of the mixer, everyone met a host of new people and newcomers began to feel more a part of the Laboratory colony.

Mrs. K. M. Wilbur has been appointed the new hostess for the M.B.L. Club this year.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
June 28	7:04	7:20
June 29	7:50	8:09
June 30	8:37	9:01
July 1	9:29	9:56
July 2	10:23	10:56
July 3	11:18	11:59
July 4	.....	12:19
July 5	12:59	1:16
July 6	1:56	2:12
July 7	2:54	3:08

In each case the current changes approximately six hours later and runs from the Sound to the Bay.

ITEMS OF INTEREST

DR. ARTHUR K. PARPART, in charge of the Physiology Course of the Marine Biological Laboratory, has been promoted from assistant to associate professor of biology at Princeton University.

DR. RITA GUTTMAN was promoted from tutor in physiology to instructor in physiology at Brooklyn College on the first of this year.

DR. CARL G. HARTMAN, of the department of embryology of the Carnegie Institution of Washington at Johns Hopkins University, has been appointed professor of zoology and head of the departments of zoology and physiology at the University of Illinois beginning September 1.

PROFESSOR ROBERT CHAMBERS left for New York on Thursday. At Washington on Saturday he will take part in a conference on shock under the auspices of the medical division of the National Research Defense Committee.

Fourteen persons attended the first meeting of the course in conversational German conducted by Dr. Olga Janowitz on June 20. The class will continue to meet weekly on Fridays between seven and eight in the evening.

MISS MARGARET HENSON is organizing a group of people interested in taking a Red Cross first aid course. Fifteen or twenty people are required to make a complete class, which will meet two hours a week for ten weeks. Anyone interested in taking the course should see Miss Henson in room 217 of the Brick Building, or Mrs. D. Morris as soon as possible.

The course in protozoology at the Marine Biological Laboratory has been discontinued.

Choral Club Rehearsals Scheduled

Persons interested in singing good music are cordially invited to join the Woods Hole Choral Club, which will hold its first rehearsal at the Canteen Building of the United States Bureau of Fisheries on Tuesday, July 1. It is sixteen years since the Club held its first meeting, and during its existence it has provided an opportunity for persons connected with the laboratory to sing worthwhile music under competent direction. This summer, as usual, the Club will rehearse for a concert which will be presented in the latter part of August.

Prof. Ivan T. Gorokhoff, Director of Choral Music at Smith College, will again direct the Club this year. There are no dues. Rehearsals are held twice a week, on Tuesdays at nine o'clock and on Thursdays at eight. While the first rehearsal will be held Tuesday, the one on Thursday this week will not be held because of the holiday.

RUFUS H. THOMPSON, teaching assistant in botany at Stanford University, was injured in an automobile accident while he was a passenger *en route* to Woods Hole. He is convalescing in a hospital in Laramie, Wyoming, from a broken leg and a cracked shoulder blade.

The absence of Mr. Thompson would have seriously interfered with the routine of the work of the Algae course, except for the fact that Assistant Professor C.-K. Tseng of Lingnan University and Mr. W. J. Gilbert of the University of Michigan had come to Woods Hole to continue their doctorate studies under the direction of Professor Taylor. They are devoting part of their time to substituting for Mr. Thompson on the laboratory staff.

On July 3rd Dr. S. C. Brooks will be a guest lecturer before the physiology class. He will talk on "Some Problems in Permeability". Dr. L. V. Heilbrunn lectured to the class this morning.

The first botany seminar was held last Thursday evening; Robert H. Williams of Cornell University spoke on the "Carbohydrate Metabolism of the Large Brown Algae."

MR. EDWARD CHAMBERS, son of Dr. Robert Chambers, married Miss Zoya Zarudnaya on June 3rd in New York. Miss Zarudnaya has been studying sculpturing in New York. They are expected to return to Woods Hole from New York in July. Mr. Chambers is a medical student of New York University.

MISS EDITH BILLINGS, who has been secretary in the Administration Office of the Marine Biological Laboratory for a number of years, was married on June 21 to Mr. James J. Reilly, local contractor, at St. Joseph's Church in Woods Hole.

Tennis Courts Reopen

Both the mess courts and the Colas courts of the M.B.L. Tennis Club are now open, and it is expected that the beach courts will be ready for play in about a week. The first court was put into condition last Saturday, two weeks earlier than last year.

Membership rates this year are $6.00 for full membership, and $4.00 for persons wishing to play only on the Colas courts. A special rate of $3.00 is being quoted to students taking the courses at the Marine Biological Laboratory. Guests may play on the courts for twenty-five cents an hour.

The President of the Club this year is Dr. D. E. Lancefield; Dr. T. K. Ruebush is Secretary-Treasurer. Albert Stunkard continues as groundskeeper.

THE SYMPOSIUM ON GENES AND CHROMOSOMES AT THE COLD SPRING HARBOR BIOLOGICAL LABORATORY

As part of its policy of fostering a closer relation between biology and the basic sciences, the Laboratory invites each summer a group actively interested in a specific aspect of quantitative biology, or in methods and theories applicable to it, to carry on their work and to take part in a Symposium at the Laboratory. The aim is that every important aspect of a given subject should be adequately represented, from the physical and chemical, as well as from the biological point of view.

The Symposium this year, June 18 - July 2, will deal with genes and chromosomes. As a rule the participants will be in residence at Cold Spring Harbor during all of the two weeks' period.

Each day's program begins at 9:30 A. M. When three papers are scheduled for the same day, the third one will be read at 2:15 P. M.

STRUCTURE OF CHROMOSOMES AS REVEALED BY OPTICAL METHODS

Wednesday, June 18th

Warmke, H. E. External morphology of chromosomes.
Nebel, B. R. Structure of plant chromosomes, with particular emphasis on the number of chromonemata.
Huskins, C. Leonard. The coiling of chromonemata.

Thursday, June 19th

Berger, C. A. Multiple chromosome complexes in animals and polysomaty in plants.

SALIVARY GLAND CHROMOSOMES

Metz, C. W. Structure of salivary gland chromosomes.
Mazia, Daniel. Enzymatic studies of chromosomes.

Friday, June 20th

Painter, T. S. Chemical studies of chromosomes.
Schultz, Jack. The evidence of the nucleoprotein nature of the genes.
Cole, P., and Sutton, E. Variation in the absorption of ultraviolet irradiation by the bands of salivary gland chromosomes.

SPONTANEOUS AND INDUCED CHANGES IN CHROMOSOME STRUCTURE

Saturday, June 21st

McClintock, Barbara. Spontaneous alterations in chromosome size and form in Zea.
Kaufmann, B. P. Induced chromosomal breaks in Drosophila.

Monday, June 23rd

Sax, Karl. Effect of irradiation on plant chromosomes.
Carlson, J. Gordon. Effects of irradiation on grasshopper chromosomes.
Fano, U. On the analysis and interpretation of chromosomal changes in Drosophila.

Tuesday, June 24th

Delbruck, M. Biophysical analysis of chromosome behavior.

MUTATIONS

Plough, Harold H. Spontaneous mutability in Drosophila.
Rhoades, M. M. The genetic control of mutability in maize.

Wednesday, June 25th

Demerec, M. Unstable genes in Drosophila.
Muller, H. J. Induced mutations in Drosophila.

Thursday, June 26th

Stadler, L. J. Comparative studies of the genetic effects of X-rays and ultraviolet radiation.
Hollaendar, Alexander. Wavelength dependence for mutation production with special emphasis on fungi.
Gowen, John. Mutation in Drosophila, bacteria and viruses.

PHYSICAL ASPECTS AND TOOLS

Friday, June 27th

Schmitt, F. O. Birefringence and viscosity as a tool for the study of submicroscopical structures.
Zworykin, V. K. Electron microscope.
Fankuchen, I. X-ray diffraction studies of biological substances.

PROPERTIES OF GIANT MOLECULES AS RELATED TO CHROMOSOME PROBLEMS

Saturday, June 28th

Mark, H. Structure and reactivity of long-chain molecules.
Fruton, Joseph S. Proteolytic enzymes as specific agents in the formation and breakdown of proteins.

Monday, June 30th

Wrinch, Dorothy M. The native protein as a megamolecule.
Greenstein, Jesse P. Physical changes in thymonucleic acid induced by proteins, salts and ultraviolet irradiation.
Stanley, W. M., and Knight, C. A. The chemical composition of different strains of tobacco mosaic virus.

Tuesday, July 1st

Claude, Albert. Particulate components of cytoplasm.
Rothen, Alexandre. Specific properties of proteins in films.
Mirsky, A. E. Some observations on protein folding and unfolding.

Wednesday, July 2nd

Rittenberg, D. The state of the proteins in animals as revealed by the use of isotopes.

CONCLUSION

Muller, H. J. Resumé and prospectives.

DIRECTORY FOR 1941

KEY

Laboratories		Residence	
Botany Building	Bot	Apartment	A
Brick Building	Br	Dormitory	D
Lecture Hall	L	Drew House	Dr
Main Room in Fisheries Laboratory	M	Fisheries Residence	F
Old Main Building	OM	Howes	Ho
Rockefeller Bldg.	Rock	Hubbard	H
Supply Dept.	S	Kahler	Ka
		Kidder	K
		Whitman	W

MARINE BIOLOGICAL LABORATORY

THE STAFF

Packard, C. director. asst. prof. zool. Dept. Cancer Research, Columbia.

ZOOLOGY

Investigation

Calkins, G. N. prof. proto. Columbia.
Conklin, E. G. prof. zool. Princeton.
Grave, C. prof. zool. Washington (St. Louis).
Jennings, H. S. prof. zool. California.
Lillie, F. R. prof. emb. Chicago.
McClung, C. E. prof. zool. Pennsylvania.
Mast, S. O. prof. zool. Hopkins.
Morgan, T. H. dir. biol. lab. California Tech.
Parker, G. H. prof. zool. Harvard.
Woodruff, L. L. prof. proto. Yale.

Instruction

Bissonnette, T. H. prof. biol. Trinity. in charge.
Crowell, P. S., Jr. asst. prof. zool. Miami.
Jones, E. R., Jr. prof. biol. William and Mary.
Lucas, A. M. assoc. prof. zool. Iowa State.
Martin, W. E. asst. prof. zool. DePauw.
Matthews, S. A. asst. prof. biol. Williams.
Mattox, N. T. instr. zool. Miami.
Rankin, J. S., Jr. instr. biol. Amherst.
Waterman, A. J. asst. prof. biol. Williams.

EMBRYOLOGY

Investigation (See Zoology)

Instruction

Ballard, W. W. asst. prof. biol. & anat. Dartmouth.
Costello, D. P. asst. prof. zool. North Carolina.
Goodrich, H. B. prof. biol. Wesleyan. in charge.
Hamburger, V. assoc. prof. zool. Washington.
Schotté, O. assoc. prof. biol. Amherst.

PHYSIOLOGY

Investigation

Amberson, W. R. prof. phys. Maryland Med.
Bradley, H. C. prof. phys. chem. Wisconsin.
Garrey, W. E. prof. phys. Vanderbilt Med.
Jacobs, M. H. prof. phys. Pennsylvania.
Lillie, R. S. prof. gen. phys. Chicago.
Mathews, A. P. prof. biochem. Cincinnati.

Instruction

Ballentine, R. fel. biol. Princeton.
Fisher, K. C. asst. prof. exper. biol. Toronto.
Kempton, R. T. prof. zool. Vassar.
Parpart, A. K. assoc. prof. biol. Princeton. in charge.
Prosser, C. L. asst. prof. zool. Illinois.
Sichel, F. J. M. asst. prof. phys. Vermont Med.

BOTANY

Investigation

Brooks, S. C. prof. zool. California.
Duggar, B. M. prof. phys. & econ. bot. Wisconsin.
Goddard, D. R. asst. prof. bot. Rochester.
Sinnott, E. W. prof. bot. Yale.

Instruction

Gilbert, W. J. grad. bot. Michigan.
Runk, B. F. D. instr. bot. Virginia.
Taylor, W. R. prof. bot. Michigan. in charge.
Tseng, C.-K. asst. prof. biol. Lingnan.

INVESTIGATORS

Allee, W. C. prof. zool. Chicago. Br 332. A 101.
Allen, Joan OM Base.
Alsup, F. W. grad. zool. Pennsylvania. Dr Attic.
Amberson, W. R. prof. phys. Maryland Med. Br 109.
Baker, Gladys E. instr. plant sci. Vassar. lib.
Baker, H. B. prof. zool. Pennsylvania. Br 221.
Baker, L. A. res. asst. phys. chem. Lilly Res. Labs. Br 319.
Ball, E. G. asst. prof. biochem. Harvard Med. lib. D 314.
Ballard, W. W. asst. prof. biol. & anat. Dartmouth. OM 40.
Ballentine, R. fel. phys. Princeton. OM 2.
Barron, E. S. G. asst. prof. biochem. Chicago. Br 110.
Bartlett, J. H., Jr. assoc. prof. theoretical physics. Illinois. OM 43.
Benedict, D. Milton Acad. (Mass.). Br 309.
Berger, C. A. prof. cytol. Fordham. Br 225.
Bernheimer, A. W. grad. bact. Pennsylvania. Br 234. D 209.
Bissonnette, T. H. prof. biol. Trinity. OM 28. (July 15).
Bliss, A. F. lect. biophysics. Columbia. Br 314. Ho 2.
Bowen, W. J. asst. prof. zool. North Carolina. Br 329.
Bradford, Audrey A. Vassar. Br 227.
Bradley, H. C. prof. physiol. chem. Wisconsin. Br 122-A.

Brill, E. R. grad. biol. Harvard. Br 217-M.
Brooks, Matilda M. res. assoc. zool. California. Br 322.
Brooks, S. C. prof. zool. California. Br 322. D 107.
Brownell, Katherine A. res. asst. phys. Ohio State. Br 111.
Budington, R. A. prof. zool. Oberlin. Br 218. (Sept. 1).
Bullock, T. H. fel. zool. Yale. Br 217-n.
Cable, R. M. assoc. prof. parasit. Purdue. Br 223.
Carothers, Eleanor res. assoc. zool. Iowa. Br 340.
Chase, A. M. instr. biol. Princeton. Br 231.
Claff, C. L. res. assoc. biol. Brown. Br 312. A 208.
Clark, E. R. prof. anat. Pennsylvania Med. Br 117.
Clark, L. B. asst. prof. biol. Union. Br 315. D 109.
Clowes, G. H. A. dir. res. Lilly Res. Labs. Br 328.
Cohen, I. res. asst. biol. New York. Br 310.
Cole, K. S. assoc. prof. phys. Columbia Med. Br 114.
Colwin, A. L. instr. biol. Queens (New York). OM 45. D 210.
Colwin, Laura H. instr. biol. Vassar. OM 45. D 210.
Commoner, B. tutor biol. Queens (New York). Br 121.
Compton, A. D., Jr. grad. zool. Yale. lib.
Conger, Martha techn. N. Y. State Dept. Pub. Health. Br 122-B.
Conklin, E. G. prof. biol. Princeton. Br 321.
Copeland, M. prof. biol. Bowdoin. Br 334.
Cornman, I. asst. zool. Michigan. K 7. Br 228.
Costello, D. P. asst. prof. zool. North Carolina. Br 123. D 202.
Crawford, J. D. Milton Acad. (Mass.). Br 309.
Crowell, S. asst. prof. zool. Miami. OM 25.
Curtis, W. C. prof. zool. Missouri. Br 335. (Aug. 1).
Dewey, Virginia C. res. fel. proto. Brown. OM 22. D 308.
Donnellon, J. A. asst. prof. biol. Villanova. Rock 3.
Donovan, Mary K. grad. biol. Villanova. Rock 2.
DuBois, A. B. Milton Acad. (Mass.). Br 309.
DuBois, E. F. prof. med. Cornell Med. Br 317.
Duggar, B. M. prof. plant phys. & applied bot. Wisconsin. Br 304. (Aug. 1).
Dumm, Mary E. grad. biol. Bryn Mawr. Br 118. D 311.
Dytche, Maryon grad. phys. Pittsburgh. Rock 6. WA.
Dziemian, A. J. fel. zool. Pennsylvania Med. Br 204.
Edwards, G. A. grad. asst. biol. Tufts. Br 217-o.
Evans, T. C. asst. prof. radiol. Iowa. Br 340. D 302.
Ferguson, F. P. asst. zool. Minnesota. Br 210.
Fisher, K. C. asst. prof. expt. biol. Toronto. OM Phys. D 214.
Forbes, J. asst. prof. biol. Fordham. Br 225.
Frasier, Doris A. instr. biol. Russell Sage. OM 44.
Frisch, J. A. prof. biol. Canisius (Buffalo). OM 39.
Gabriel, M. L. asst. zool. Columbia. Br 314.
Garrey, W. E. prof. phys. Vanderbilt Med. Br 215.
Gates, R. R. prof. bot. London (Ont.). lib.
Gilbert, W. J. grad. bot. Michigan. Bot. Dr 6.
Gilman, L. C. grad. zool. Hopkins. OM 36-b.
Glancy, Ethel A. tutor biol. Queens (New York). OM 36.
Goldin, A. grad. zool. Columbia. Br 313.
Goodrich, H. B. prof. biol. Wesleyan. Br 210. D 204.
Goulding, Helen J. grad. phys. Toronto.
Grave, C. prof. zool. Washington (St. Louis). Br 327.
Groupé, V. grad. immun. Pennsylvania. Br 234.
Guttman, Rita instr. phys. Brooklyn. Br 110.
Haas, W. J. M.I.T. Br 116.
Hager, R. P. res. asst. zool. Pennsylvania. Rock 2.
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Runk, B. F. D. instr. bot. Virginia. Bot 26. D 110.
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Shaw, Myrtle senior bact. N. Y. State Dept. Health. Br 122-B. D 303.
Shelanski, L. grad. zool. Pennsylvania. OM Base. Dr 15.
Shelden, F. F. instr. phys. Ohio State. Br 111. K 24.
Sichel, Elsa K. head sci. dept. State Normal Sch. (Johnson, Vt.). OM 4. K 8.
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Slaughter, J. C. grad. asst. zool. Iowa. Br 340.
Slifer, Eleanor H. asst. prof. zool. Iowa. Br 217-a. D 203.
Smith, J. A. instr. phys. & pharmacol. Chicago Med. Br 6.
Solberg, A. N. asst. prof. biol. Toledo. lib. (Aug. 3).
Speidel, C. C. prof. anat. Virginia. Br 106. D 315.
Steinbach, H. B. asst. prof. zool. Columbia. Br 313.
Stern, K. G. res. asst. prof. physiol. chem. Yale Med. Br 110. (Aug. 1).
Stokey, Alma G. prof. bot. Mt. Holyoke. Bot 1.
Stowell, R. E. res. asst. Barnard Hospital (St. Louis). lib.
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Thivy, Francesca grad. bot. Michigan. Bot.
Trinkaus, J. P. grad. zool. Columbia. OM 41.
Troedsson, Pauline H. grad. zool. Columbia. Br 314.
Trombetta, Vivian V. instr. bot. Smith. Bot 1.
Tseng, C.-K. asst. prof. bot. Lingnan (China). Bot 1. Dr 6.
Wald, G. instr. biol. Harvard. Br 318.
Walker, R. instr. biol. Rensselaer. OM 38.
Warner, E. N. instr. zool. Ohio State. OM 36.
Waterman, A. J. asst. prof. biol. Williams. OM 26. (July 20).
Watterson, Ray asst. biol. Hopkins. OM 41.
Weisiger, J. R. grad. fel. physiol. chem. Hopkins. Br 224. D 111.
Wenrich, D. H. prof. zool. Pennsylvania. Br 219. (Aug. 1).
Whiting, Anna R. guest invest. zool. Pennsylvania. Rock 2.
Whiting, P. W. assoc. prof. zool. Pennsylvania. Rock 2.
Wichterman, R. asst. prof. biol. Temple. Br 217-h.
Wiercinski, F. J. res. asst. zool. Pennsylvania. Br 220. K 7.
Wilbur, K. M. res. assoc. biol. New York. Br 342. K 12.
Willier, B. H. prof. zool. Hopkins. Br 324. A 302.
Wilson, W. L. grad. zool. Pennsylvania. OM Base.
Wolf, E. A. assoc. prof. biol. Pittsburgh. Rock 7.
Wolf, Opal M. asst. prof. zool. Goucher. Br 122-C. A 206.
Woodruff, L. L. prof. proto. Yale. Br 323.
Woodward, A. A. grad. asst. biol. New York. L 21. Dr 3.
Woodward, A. E. asst. prof. zool. Michigan. (Aug. 20).
Wrinch, Dorothy lect. chem. Hopkins. lib.
Yntema, C. L. asst. prof. anat. Cornell Med. Br 317. D 310.
Young, W. C. assoc. prof. primate biol. Yale. lib. (Aug.).
Zarudnaya, Katya Radcliffe. OM 36. H 6.
Zinn, D. J. grad. zool. Yale. OM 38.
Zorzoli, Anita asst. zool. Columbia. OM 36. H 7.
Zweifach, B. W. res. assoc. biol. New York. Br 310.

STUDENTS

Abbott, C. C. Haverford. bot.
Algire, G. H. grad. anat. Maryland Med. phys.
Bayard, Ellen B. U. of Connecticut. bot. H 8.
Bergstrom, W. H. Amherst. emb.
Birmingham, L. W. Rochester. emb. Dr 3.
Bull, Nancy B. Wellesley. bot. H 2.
Burns, J. E., Jr. grad. asst. biol. Wesleyan. phys.
Coe, F. W. Ohio Wesleyan. phys. Ho 6.
Cole, R. M. teach. fel. biol. Harvard. emb. Ka 3.

Covalla, Miriam J. instr. biol. Seton Hill (Greensburg, Pa.). emb. H 1.
Crumb, Cretyl Wellesley. emb. H 1.
Deyrup, Ingrith J. Barnard. phys.
Egan, R. W. res. asst. biol. Canisius (Buffalo, N. Y.). phys. Dr 2.
Eldred, E. asst. biol. Northwestern. emb. Dr 3.
Enzenbacher, Jean A. Smith. bot. W B.
Frantz, Ruth E. Elmira. bot. D 307.
Gibbs, Elizabeth Wheaton. emb. W C.
Gordon, H. T. teach. fel. biol. Harvard. phys. Ho 1.
Green, J. W. Davis-Elkins. phys.
Gregg, J. R. asst. biol. Alabama. phys.
Gross, J. B. De Pauw. emb.
Harrison, R. W. grad. asst. biol. Wesleyan. phys. K 7.
Hartmann, J. F. asst. zool. Cornell. phys. Dr 1.
Hasse, G. W. grad. asst. zool. Illinois. emb.
Hendricks, Anne L. Cincinnati. emb.
Henry, Jane E. Gettysburg. phys. H 3.
Hollister, Patricia Sarah Lawrence. emb.
Hopkins, Marjorie G. grad. asst. biol. Mt. Holyoke. emb. H 7.
Jacobs, J. teach. fel. biol. New York. phys. Dr 1.
Josephson, N. Wesleyan. emb. K 5.
Karczmar, A. G. grad. biol. Columbia. emb.
Katz, Elaine J. Goucher. bot. D 307.
Keezer, W. S. Indiana. phys. K 15.
Kezer, L. J. instr. biol. State Teachers (Newark, N. J.). emb. K 9.
Kieffer, R. F., Jr. Franklin and Marshall. emb. Ho 2.
Kirkpatrick, Elizabeth M. Connecticut College. emb.
Lambie, M. W. Harvard. emb.
Lein, J. C.C.N.Y. phys. Ka 22.
Lerner, Eleanor D. grad. biol. Brooklyn. emb. W D.
Leuchs, Augusta V. H. A. grad. biol. Radcliffe. bot. H 2.
Linthicum, Anne H. Goucher. emb. D 106.
Lorenz, P. B. Swarthmore. phys. Ho 3.
Martin, R. G. Harvard. emb. Dr 15.
Martin, T. S. Oberlin. emb. K 15.
Medlicott, Mary grad. asst. biol. Mt. Holyoke. emb. H 7.
Morgan, T. W. Washington and Jefferson. emb. Dr 10.
Mothes, Arlene M. Massachusetts State. emb. W E.
Muchmore, W. B. Oberlin. emb. K 7.
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Perkins, Patricia J. grad. chem. Cincinnati. phys. W F.
Plough, I. C. Amherst. emb.
Power, M. E. grad. asst. zool. Yale. emb. Ka 2.
Regnery, D. C. lab. instr. biol. Stanford. emb. K 9.
Reiner, E. R. lab. asst. biol. Alabama. emb.
Rollason, H. D., Jr. grad. asst. biol. Williams. phys. Dr 7.
Rosenblum, E. D. Brooklyn. phys. K 10.
Royle, Jane G. grad. biol. Bryn Mawr. emb.
Salvin, S. B. grad. asst. biol. Harvard. bot. Ka 3.
Saunders, Grace S. teach. fel. biol. New York. emb. H 9.
Schepartz, B. Ohio Wesleyan. phys. Dr 5.
Schlosser, Adele P. Vassar. phys.
Smith, F. B. prof. biol. Florida. bot.
Smithcors, J. F. Rutgers. emb. Ka 2.
Stanton, Constance L. Bryn Mawr. bot. W G.
Stenger, F. R. prof. biol. St. Mary of the Lake Seminary (Mundelein, Ill.). bot.
Stern, J. R. res. asst. med. Toronto. phys. K 6.
Svihla, G. res. asst. zool. Illinois. emb. Ho 2.
Thorne, R. F. Dartmouth. bot. Ka 21.
Timanus, Alice L. Converse. emb. D 106.
Tuttle, L. Constance grad. asst. biol. Mt. Holyoke. phys. W H.
Waldron, Jacqueline M. American. bot. H 3.
Wasserman, E. asst. biol. Wesleyan. emb. K 5.
Weiner, H. M. Harvard. phys. Dr 1.
Williams, R. H. instr. bot. Cornell. bot. D 208.
Zimmerman, G. L. Swarthmore. phys. Ho 3.
Zingher, J. M. C.C.N.Y. emb. K 10.

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Weisiger, J. R. fel. physiol. chem. Hopkins. Br 224. D 111.

Apparatus and Technical Service

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Bridgman, Jane grad. biol. Harvard. photographer. H.
Graham, J. D. Pennsylvania. glass blower. Br 22.
Lefevre, Dorothy E. sec. Br 1.
Liljestrand, Robert S. Br 3.
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Cannon, F. janitor.
Fink, F. janitor. Ka 4.
Genther, T. janitor. Ka 4.
Hemenway, W. C. carpenter.
Kahler, R. S. asst.
Larkin, R. janitor.
Larkin, T. E. supt. Br 7.
Roth, P. janitor. Ka 1.
Spier, R. janitor. Ka 4.
Tawell, T. E. head janitor.
Taylor, R. night mechanic. Dr Attic.

Travis, R. F. mail.
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Wynn, J. fireman.

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Glass, B. A & M College (Texas). collector. Ho 9.
Goodrich, A. animal house. Ho 5.
Gray, M. B. collector.
Harman, Grace sec. WH.
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Kahler, W. E. collector.
Kyllonen, A. Harvard. collector. Ho 4.
Kyllonen, D. collector. Ho 4.
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Mandly, H. second officer.
McMurray, F. S. captain.

"Anton Dohrn"

Atheran, E. captain.
Daggett, R. engineer.
Poole, S. mate.

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Schroeder, W. supt.

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PROTOPLASMIC STREAMING

DR. DOUGLAS A. MARSLAND

Assistant Professor of Biology, Washington Square College, N. Y. University

High hydrostatic pressure, the type of pressure which reaches great intensity in the oceanic depths, has been applied artificially to various living tissues and has proved a useful tool for analyzing some of the reactions which take place in the protoplasma of living cells, — reactions which provide the energy for the characteristic activity of the cell. One type of activity, namely protoplasmic streaming, appears to be especially sensitive to the effects of pressure. The streaming of the protoplasm of various Amoeboid cells during active locomotion, the protoplasmic currents which occur at the time that animals are dividing, the cyclic flow of protoplasm which is characteristic of so many plant cells, and the ebb and flow of pigment granules back and forth in the fine branches which radiate out from the pigment cells of the skin of many fishes,—all of these forms of streaming are re- (*Continued on page* 31)

M. B. L. Calender

TUESDAY, July 8, 8:00 P. M.

Seminar: Physiological seminar in which the following investigators will take part: A. J. Dziemian, Rita Guttman and Herbert Shapiro.

FRIDAY, July 11, 8:00 P. M.

Lecture: "The Integration of Neurology and Psychology," Dr. K. S. Lashley, Research Professor of Neuropsychology, Harvard University.

HOW FEATHERS ARE MADE

DR. FRANK R. LILLIE

Professor of Embryology, Emeritus, University of Chicago

I. Introduction

If this question could be fully answered within the limits of present scientific principles and methods we would have much deeper insight into problems of physiology, genetics and development. But our knowledge is still very incomplete; and of the little that we know I shall be able to tell you only a part of my own work, done originally with the aid of Dr. Mary Juhn and recently of Mr. Hsi Wang.

Each feather is an autobiographical record written in the course of its life history—a sort of diary, in fact. To read it one needs a Rosetta Stone, and this is furnished by study of its growth and development. By plotting rates of axial growth with reference to the location of simultaneous reactions in the germ (isochrones) it is possible to relate any definitive part of the feather to its place and time of origin, and hence to the conditions of determination when these are

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THE UPLANDS OF NAUSHON, LOOKING TOWARD TARPAULIN COVE

For years it has been customary for the various classes of the laboratory to take their annual picnic on the beach near the lighthouse seen in the background. The Cove opens out on Vineyard Sound.

known. By virtue of these properties, for instance, relations between rates of growth and thresholds of reaction have been established, and also between gradients of threshold and structure and pattern in the individual feathers.

Regenerating feathers of the fowl are excellent material for study. They can be made available at any time by plucking, and during most of the period of regeneration they are extremely sensitive indicators to alterations of physiological condition, both natural and such as may be induced by controlled experiments.

Experiments with reactions of saddle and neck hackle feathers of Brown Leghorn males to injections of thyroxin illustrate sensitiveness of reaction of growing feathers. (Lantern slides). A single injection of 0.5 mg. in a bird weighing 1800 grams produces a spindle-shaped black mark adjacent to the rhachis, the length of which indicates the duration of reaction, and the form of which indicates absorption to the center of the spindle and excretion thereafter of the excess thyroxin. The threshold of reaction is least immediately next to the rhachis and becomes increasingly higher towards the apex of the barbs. Single injections of 1.0 mg, 1.5 mg, 5.0 mg, etc., produce successively broader areas until the margin of the feather is reached with 5.0 mg. There is thus a gradient of threshold along the axis of each barb to thyroxin.

The stated action of thyroxin is on the melanoblasts, which rapidly grow into melanophores, producing pigmentation otherwise absent.

This method may be used also to produce interesting patterns, as illustrated by neck hackle feathers in which successive injections of 1.0 mg. of thyroxin every seventh day produces a repeated pattern, and 1.5 mg. every sixth day produces a similar repeated pattern but broader and closer.

II. General

In order to give an account of the experiments which form the principal subject of this lecture it is necessary to introduce a short account of the anatomy and development of the feather. Feathers from different tracts of one bird differ from each other much as animals of two different species do. The account therefore specifies the feathers of the breast and saddle tracts of the Brown Leghorn male. Study of particulars is necessary in order to reach sound general conclusions.

1. Anatomy

The feather is not formed by budding and branching as are, for instance, "feathery" hydroids (such as Pennaria), but is composed of parts separately formed and secondarily assembled. It can therefore be taken apart like a machine. These parts are the shaft (rhachis), the barb stem, and the barbules. The barbules do not grow out of the barb stem. The barbs do not grow out of the rhachis.

The rhachis is formed of a central and two lateral components. The lateral components carry the barbs and are, indeed, composed of the union of modifications of the bases of barbs called the "barb petioles" which are bound together by long keratin fibers originating within the petioles. (Illustrated by lantern slides.)

2. Sketch of Normal Development

The feather develops from a "papilla" situated at the bottom of a follicle six to eight mm. deep in the case of the breast feather opening on the surface of the skin. It emerges from the follicle in the form of a cylinder covered by a stiff sheath of keratin. As the feather grows the barbs and rhachis burst through the sheath and extend far beyond it. The base of the growing feather is always in the form of a cylinder.

A longitudinal section of the feather cylinder (illustrated by lantern slides) shows the relationships of the growing parts of the feather, all of which arise from a thick ring of embryonic cells, the collar, the central opening of which (umbilicus) is occupied by the papilla.

When the feather is plucked the papilla remains behind in the bottom of the follicle, consisting of a massive mesodermal core and a thin covering of a single layer of ectodermal cells from which the new feather is to be formed.

III. Operations on the Papilla

The purpose of this lecture is not a general review, but to present an account of experiments in which old methods of experimental embryology have been applied for the first time to the development of feathers.

The method of operating is to open the follicle so as to expose the papilla, and operate on the papilla with the finest iridectomy scissors. The follicle heals promptly.

There are various kinds of operations that can be performed: (1) transplantation experiments, in which the papilla is completely removed from

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the follicle and transferred to some other site on the host; (2) defect experiments, in which parts of the papilla are removed, leaving the remainder within the follicle to develop; (3) isolation experiments, in which the papilla is divided longitudinally in determinate planes, and both parts left to develop within the same follicle; and (4) recombination experiments, in which part of the papilla from one follicle is removed and replaced with a supplementary part removed from another papilla.

1. Transplantation Experiments

The follicle from which a papilla has been removed does not regenerate a new papilla or feather. The papilla is the indispensable requisite for the formation of a feather. The feather papillae are tract-specific: if one takes a saddle papilla and implants it within a breast follicle from which its own papilla has been removed, the saddle papilla will produce a saddle feather in the breast. These feather germs or papillae behave just as specifically as different kinds of eggs. Wherever papillae are transplanted within the organism, there they produce the kind of feather peculiar to the site of origin.

The papilla has a definite bilateral organization. If one dissect a papilla entirely free from all its attachments in the follicle, and rotate it 180° within the follicle, an upside-down feather results, i.e., ventral surface up and dorsal surface down.

The papilla has no land marks on it like the frog's egg and so the operations must be made with reference to the position of the papilla within the follicle. It lies obliquely inclined with dorsal surface up, ventral surface down. If one removes the ventral half by frontal bisection, the dorsal half will form a normal feather; and if one divides the feather longitudinally and sagittally, a normal feather will also develop from a lateral half. Dorsal and lateral halves are thus completely regulable.

The situation is entirely different in the case of ventral halves. When the dorsal half of the papilla is removed the remaining ventral half produces one of two kinds of feathers, i.e., either "tuft" feathers in which there is no rhachis and the feather consists of a series of independent barbs, or "half-vane" feathers in which one lateral half is formed in a fairly normal fashion and the other lateral half is similar to tuft feathers except that the barbs adjoining the half-rhachis are attached by their apexes and the bases are free. The production of the tuft feather is explained by the *complete* removal of the dorsal half of the papilla which is necessary for formation of a rhachis; and the half-vane feather is explained by slight deviations right and left that leave narrow margins of the dorsal half of the papilla, each inducing a half-rhachis. Correspondingly, it is found that about half of the half-vane feathers are right-handed and half left-handed.

There is a clear analogy with the amphibian egg in respect to the behavior of dorsal and ventral halves respectively.

Feathers with defects confined to the apex may be produced by amputation of the apical half of the papilla. These are to be interpreted as produced not by loss of a prospectively limited segment of the papilla, but by the delay in formation of the rhachis caused by the experiment, which produces in the apex of the feather conditions similar to the tuft and half-vane feathers.

2. Isolation Experiments

Twin feathers may be produced by dividing the papilla sagittally by a single cut and leaving the lateral halves in place. If each half develops independently two normal and complete feathers are produced in a single follicle. But if the halves fuse together to a greater or less extent, as they usually do, twinning is confined to the apex of the feather. Successive generations of feathers from the same follicle produce types of separate or conjoint twin feathers similar to the first generation.

3. Recombination Experiments

Interesting *chimera feathers* may be produced by combining right and left lateral halves of papillae within the follicle of one of them. This was illustrated specifically by chimerae between breast and saddle feathers in which one half-vane was saddle type and the other half-vane breast type.

IV. Discussion

In all of the experiments described above the defective papillae continue to produce the same types of defective feathers in successive generations. In some cases as many as five successive generations have been recorded. There is a very pronounced lack of regulative ability on the part of operated papillae.

From the experiments it follows that the massive mesodermal core of the papilla acts as inductor on the ectoderm and determines the establishment of a dorsal field in the ectoderm, within which the rhachis develops. Without this the development is always abnormal.

From various lines of evidence it was concluded that the period of induction by the mesoderm is brief, and that thereafter the ectoderm constitutes a self-regulating system. The effects of the operations within the definitive feather are due to disturbances of the inductive action on the ectoderm. This inductive action is general, not specific or mosaic.

Out of hundreds of abnormal feathers produced experimentally no two are alike. However, all individual feathers produced in the defect experiments fall into the three main classes, "tuft"

feathers, "half-vane" feathers, and feathers with apical defects only. These are characterized by defects of the rhachis.

Three kinds of barb defects occur in all three classes: branched, united and bundled. In the half-vane feathers, in addition to these barb abnormalities, there occur also on the defective side of the half-rhachis reversed barbs which are attached by their apexes with their bases free.

Branched barbs are due to fusion of the growing barbs to form a single base which continues to grow as one, owing to disturbance of the regular tangential movement. United barbs, i.e., with single apex and two or more bases, are due to division of the growth center of the base, each continuing to grow independently. If the fusion of the growth center that produces branching is followed after a time by division of the common base, "bundling" results. Reversed barbs occur only in connection with a half-vane and are due to the formation of a new apex adjacent to the half-rhachis and fusion of this apex with the out-growing rhachis.

From all this it follows that the rhachis is responsible for the normal organization of the feather. It attracts barbs from both sides and is thus responsible for the formation of the ventral triangle to which formation of new barbs is confined in the normal development. It induces the formation of petioles on barbs that become attached to it and thus terminates the growth of the barbs. It carries the completed barbs apically by its own growth and thus in general preserves the sequential order of barbs. It also determines the distinction between distal and proximal barbules; and finally, it is to be noticed that each lateral half of the rhachis operates independently, as is shown by the half-vane feathers.

The analysis of morphogenesis of the feather is then briefly as follows:

1. The dorsal half of the papilla alone has the capacity of inducing the dorsal field in the ectoderm, and this is the main morphogenetic function of the papilla.

2. The ectoderm of the papilla is capable of forming barbs which grow independently; but the mesodermal induction is necessary for the determination of the rhachis which controls the normal behavior of the barbs.

3. The assembly of barbs on the rhachis occurs after their independent differentiation. The abnormalities produced are in accordance with the principles of the special mechanism of development.

Reference: Lillie, F. R., and Wang, Hsi. 1941. Physiology of development of the feather. V. Experimental morphogenesis. Physiol. Zool., 14:103-134.

(This article is an abstract of a lecture, illustrated with lantern slides, presented at the Marine Biological Laboratory on June 27.)

INFORMAL NOTES ON *EULIMA* EMBRYOLOGY BY THE EMERITUS CURATOR OF THE M. B. L. MUSEUM

GEORGE M. GRAY

(Continued from last issue)

On August 10th the *Thyone* having gone bad and no *Eulima* eggs that I could discover, I threw out the *Thyone,* gave the *Eulima* a clean bill of fare (sea water) and left them. The dish was covered with a glass plate, and they were alone.

This morning, on looking at these same *Eulima* I discovered three fine-looking bunches of *Eulima* eggs. These had been laid between the time I changed them and this A. M., probably during the night. So far as I can prove this is the first real egg-laying of this particular lot of *Eulima* since they were brought to me on July 31st and August 2nd. This is the second time I have had *Eulima* lay after being on *Thyone,* though I think that not all the *Eulima* went on the *Thyone,* but it would be interesting to see if any more are laid by these same snails.

At 5:30 P. M. I found two more egg capsules in the dish. The last two capsules laid since morning. Now five in all.

August 12. About 8 o'clock A. M. this morning there were four more egg capsules of *Eulima* in this same dish. This makes nine *Eulima* egg capsules in this dish, up to this morning. Still keep a glass over them. There are nine *Eulima* in the dish. About all the eggs are laid on the sides of the dish, the majority well up.

August 13 — 9:15 A. M. Thirteen egg capsules. Some are in veliger stage. These thirteen egg capsules are from the nine *Eulima* last collected (July 31st and August 2nd).

August 14 — 8 A. M. Two additional egg capsules were in the dish of the *Eulima.* This makes fifteen altogether. A few of the older ones of this lot are in the veliger stage.

August 16. No eggs from the nine *Eulima.*

September 1. No real good typical egg capsules from any *Eulima* on hand since last recorded date. On August 31st I put a lot of the old *Eulima* in one finger-bowl and put a *Thyone* in

with them, to see if the change would be any encouragement to egg-laying. A number of the oldest *Eulima* have died before now.

About August 18th, three new freshly collected *Eulima* were brought to me. They were in a four-ounce jar. I left them in the jar and changed or partly changed the water, but no eggs up to August 31st. That day I changed the water more thoroughly, leaving them in the same jar.

On September 1st about 8 A. M. I found one fresh laid egg capsule probably laid late yesterday or early this A. M.

September 2. No more eggs from the three *Eulima* collected the 18th of August. Yesterday four fresh collected *Eulima* were brought in, but I did not get them until this A. M. Put them in a vessel of sea water and will hope for eggs. No eggs from *Eulima* which I put in with a *Thyone* yesterday. Everything else status quo.

September 3 — about 9:20 A. M. The egg capsule from the *Eulima* of September 1st had a few separated individual veligers slowly moving about. No fresh eggs.

September 4 — 8:30 A. M. More and faster moving veligers separately on the go this A. M. No new eggs and no eggs from the *Thyone Eulima.* Took *Thyone* out, added new sea water and await results. No eggs from any others.

September 5. The veligers in the single egg capsule of the three *Eulima* of August 18th were moving around to some extent within the jelly mass, this A. M. At 5:00 tonight they were much livelier but none have come through the surrounding jelly walls.

September 6. About same conditions prevail with *Eulima* as above.

September 7 — 9:00 A. M. Veligers moving more rapidly, but all are within the surrounding walls of the egg capsule. All other *Eulima* are same as yesterday.

September 16. Eulima veligers came through the walls this A. M. This means that it took from Sept. 5 to Sept. 16 for the larval *Eulima* to develop enough to come out of the capsule and swim around in the open sea. Some *Eulima* which I gave Dr. Alice Russell laid some eggs on *Thyone.* This I did not observe at all, though they might do so in their natural outdoor haunts. The *Eulima* seemed to get more pep and act better after a little sojourn on the *Thyone.*

So far August has been the month which I have found to be ideal for egg laying of *Eulima,* though they may breed in July, but have had no time to investigate that early, though I hope to do this next year.

Additional Observations

February 28, 1941. Since late last Fall I have been carrying something like 12 to 15 *Eulima olcacea* alive to date in a finger-bowl. I changed the water nearly every day I was at the Laboratory. There were times when they did not get a change of new water for 4 or 5 days, owing to a cold which kept me home. Also there were Sundays when I did not go to the Laboratory and some few other times, but most of the time they had a daily change. I usually kept the bowl nearly covered with a glass plate.

I think I have kept with them most of the time a live sea cucumber (*Thyone briareus*) of which they seem to be quite fond. I think I am now on the third one of these for the winter. The *Eulima,* when a *Thyone* is placed in a vessel with them, will begin to gather on it and in a few hours all or nearly all the snails will have attached themselves to the *Thyone,* on which they are partially parasitical. In changing the water I simply carefully poured off the water and either let new sea water run in until the dish is full or dip the bowl under water and thus fill it, sometimes making a double filling. In this way no true cleaning has been done on the inside of the bowl. Whatever was loose in the bowl went out when the water was poured off.

This morning I wondered if perchance there could be any eggs laid during their stay in the Laboratory during the winter. Much to my surprise I found something like a dozen egg capsules, some several days old and some only a day or two.

March 1. I took out the egg capsules, placing them in another and smaller glass dish. In this way I could tell whether any new eggs were laid in the original finger bowl, and also could tell when the larval stages came through the walls of the egg capsule out into the water and really start on their individual careers.

In the younger of these capsules the individuals lay closely packed together within the jelly-like walls of the capsule. As they grow older they begin to separate and start swimming about, but inside the walls of the capsule which seems to enlarge. It takes several days before they are ready to come out.

This morning I found three egg capsules laid near the surface of the water on the side of the bowl, so close that they seemed to touch one another. A second mass was on the bottom of the bowl, but I was not sure but what I had overlooked it in the previous lot.

On March 3rd or 4th I found five newly laid egg capsules and on March 5th six more of them, laid during the night. Nearly all, I believe, are laid at night.

March 6. Four more egg capsules were found on the sides of the finger-bowl this morning. This makes fifteen fresh capsules since the 3rd. Those of February 28 are still going.

March 8. Looked carefully this morning and decided two more egg capsules had been laid since the sixth. There are just 16 *Eulima* in the bowl with the eggs, proving that one snail at least must have laid twice. The veligers in the lot of February 28 are still swimming well, but all inside the jelly. A few of the older ones have no movement and seem dead; possibly the water got too warm. I don't think these snails lay eggs in winter in their natural habitat. I take it that the exceptionally warm temperature of the room started them laying, though of course it is possible that *Eulima* might lay at this season as some of the Nudibranchs do. But I have my doubts about *Eulima* doing so.

March 10. This morning the egg capsules had increased to 26 at least. This means that at least nine have been laid since the 8th.

The whole number are in the finger-bowl with the *Eulima* and the *Thyone.* I cannot say that any egg capsules have strictly been laid on *Thyone,* but nearly all have so far been laid on the sides of the bowl, a few on the bottom.

March 13. I took all of the *Eulima* and the *Thyone* from the finger-bowl this P. M. and put them all in another finger-bowl of clean sea water, leaving the egg capsules in the bowl in which they were laid.

I have three egg capsules with veligers alive in them. These were of the lot laid in early March. Somehow they get about a certain age and die off without leaving the egg capsule. The water gets quite warm during the night and the water I use in changing is cold. First hot and then cold may have a pernicious effect, though at first they liven up when cold fresh sea water is given them. Squeezed some veligers from a capsule this P. M. They seemed to want to burrow in the bottom of the bowl. It must be that in their outdoor habitat they would naturally burrow in the sand or mud.

Since putting the *Eulima* and *Thyone* in a clean bowl they have laid nine capsules of eggs. (This is up to March 24.) Four more laid two or three days after the change, and the others since. Have still a few of the veligers from the older lot living on March 24 (inside the capsule).

* * *

After this there seems to be a blank for I did nothing further with the lot except to put them in one finger-bowl and set them in my salt water sink, and now and then letting a little salt water run on them. Thus time passed on until this A. M., June 23rd. On looking at the *Eulima* today which I had put in a finger-bowl in the salt water sink some weeks ago I found eleven egg capsules in different stages, some quite young, some in the veliger stage. Evidently these *Eulima* had been in the sink since last March, and were not in running water. The only change they had was turning a small sea water hose into the bowl for a few seconds every other day or so, but I did not examine the snails or even look in the dish in all the time they were left in this condition. So I was surprised to find the capsules. There were seven dead shells of *Eulima,* and five live snails. I cleaned the dish, leaving the eggs and took out the dead snails' shells and put back the live snails with the egg capsules in the clean sea water, and they are still there.

This ends for a time the history of this little gastropod mollusc. There is much more to learn about its later larval development, how the shell is formed, etc. This we hope to learn later.

* * *

Since the above article went to press I have made the following observations. A collection of 10 *Eulima* was brought to me on June 25th. I put them in a clean finger-bowl of sea water. On the 26th there were no eggs laid.

On June 27th there were 8 egg capsules.
On June 28th there were 10 egg capsules.
On June 29th there were 21 egg capsules.
On June 30th there were 39 egg capsules.
On July 1st there were 52 egg capsules and one dead *Eulima.* I think the eggs were laid by the 9 as the dead one had probably been dead when brought in with the other nine. On July 2nd there were 86 egg capsules with the nine living *Eulima.*

This extends the breeding range for practically another month and perhaps *Eulima* may even breed in May. This will have to be ascertained another year. There is conclusive proof that each snail lays several egg capsules, for here are 86 egg capsules, and only nine snails for the job.

PROTOPLASMIC STREAMING

(Continued from page 25)

tarded to an equal extent and are finally abolished as the pressure is gradually raised to about 5,000 lbs. per square inch.

The least common denominator in these experiments appears to be that pressure has a liquefying action upon gelated parts of protoplasm generally. Or, to state things conversely,—high pressure inhibits gelation processes which normally must occur in cells when the streaming of the more fluid protoplasm is being motivated. In any event, it has been found that the extent to which the streaming is inhibited corresponds ex-

actly to quantitative measurements of the curtailment of the gelation processes.

An interesting assemblage of apparatus was necessary for the experiments. The pressure pump was constructed from a hydraulic jack of the type ordinarily used for lifting of heavy trucks. The thick-walled pressure chamber was made of stainless steel and provided with heavy glass windows. Since an ordinary microscopic objective would not be suitable for viewing tissues through so thick an intervening layer of glass, a special objective, with a working distance of more than half an inch, was necessary. This objective, used with an inverted type of microscope, gave good images at a magnification of 600 diameters. And lastly, the measurements on protoplasmic fluidity required a special centrifuge-pressure chamber. A suitable valve permitted no loss of pressure, even after the chamber, having received its charge from the pump, was disconnected and placed in the centrifuging equipment.

(Summary of paper presented at "The Symposium of Protoplasmic Structure" at the meetings of the American Association for the Advancement of Science, December 30, 1940.)

GRAFTING OF LIMBS IN PLACE OF THE EYE IN *AMBLYSTOMA*

Dr. Jean Piatt

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When a forelimb rudiment is grafted to the head region of salamander embryos, the resultant limb is usually well developed and capable of movement. The range and vigor of movement in the transplant vary considerably from case to case, dependent upon the specific anatomical relations existing between graft and host and upon the particular region of the head involved. A correlation of movement in the transplant with that of other muscle groups usually occurs, but the reason for this phenomenon is not entirely clear. Particularly is this the case when limbs have been transplanted in place of an eye.

Nicholas ('29, '30, '33) made homoplastic and heteroplastic forelimb grafts to the eye region in *Amblystoma*. In general, the movements of such limbs were found to be rather poor but more distinct in some cases. Some of the transplants exhibited individuated movements of the various parts. Nicholas states that when movement of a limb occurred, it was always in coordination with ocular movements of the contralateral eye.

In order to prevent the normal appearance or regeneration of the eye muscles, Nicholas cleaned the wound of all mesenchyme cells down to the wall of the brain. Although he does not actually state that the eye muscles were eliminated in the specific animals in question, it is assumed from a supplementary experiment that such was the case. The transplant in a number of cases was innervated partly by eye muscle nerves, chiefly the oculomotor. Nicholas himself states that in the event eye muscles should regenerate and insert on the base of the limb, the experiment would be vitiated, since the eye muscles themselves, not the eye muscle nerves, would be causing the coordinate movements. Nicholas interprets his results as an argument against Weiss' Resonance Theory, or theory of Homologous Response.

Weiss ('36) in an analysis of Nicholas' work makes the suggestion that in those cases which demonstrated movement synchronous with the eye some of the eye muscles might still be present and insert on the limb, thus accounting for the coordinate movements observed by Nicholas. In order to test this Weiss transplanted larval limbs in place of the eye and found that when the already developed eye muscles were not cut away in the operation they found new insertions on the transplant. The movement of these limbs was extremely weak and stereotyped, and the limb moved only as a whole. Weiss concluded that such an arrangement of the eye muscles might possibly explain the seeming discrepancy between his theory of Homologous Response and that of central coordination postulated by Nicholas.

It has been the purpose of this investigation to attack this problem anew, repeating the experiments of Nicholas but in a slightly altered form and to make a careful examination of both limb movement and the anatomy of the region involved.

Forelimb rudiments were grafted in place of the eye in embryos of *Amblystoma punctatum*. In series LHR-1 only the eye and covering ectoderm were removed; in series LHR-2 the same operation was performed but in addition the wound was cleaned of all mesenchyme cells down to the brain wall. Observations were made on the amount and type of limb movement and the full grown larvae were then sectioned and a study was made of the muscles and nerves in the region of the transplant.

No significant difference between the two series was revealed, either in movement or anatomy of the region involved. Eye muscle tissue was found to be present in every case studied, and individual muscles could often be identified. The eye muscles either inserted directly on the cartilage of the transplant or in its immediate vicinity. It is

thought that contraction of these muscles would account for the feeble, stereotyped movements observed in the grafted limbs and for the fact that the limb movements were nearly always correlated with movements in the opposite, intact eye. Limbs were always innervated by the opthalmic branches of the V-VII complex, and never by the eye muscle nerves directly, if at all.

My experiments sustantiate the contention first expressed by Weiss, that when limbs are put in place of an eye, their movement is extremely weak and stereotyped; further, that it is difficult to remove all the eye muscles in such an operation and that the coordinate movement of the limb with the opposite eye is caused by the remaining eye muscles gaining an insertion onto the base of the transplant. If this is so, then the coordination of limb with eye in such cases does not mean that non-homologous muscles are functioning in a homologous response because of innervation by nerves of an identical nature.

(This article is a summary prepared for the press of a paper presented before the American Society of Zoologists at the meeting of the American Association for the Advancement of Science on December 30, 1940.)

DEPARTMENT OF CHEMICAL SUPPLIES AND SCIENTIFIC APPARATUS

Standard Solutions:

The Chemical Room has charge of the ordering, storing, and distribution of ordinary glassware, simple laboratory apparatus, reagents, and chemicals. Drugs, dyes, and certain rarer compounds owned by the Laboratory, are all kept in the Chemical Room, #8, Brick Laboratory Basement. These may be used by investigators and classes during their work at the Laboratory.

Supplies to be used elsewhere than at this Laboratory are not furnished by the Chemical Room. All such, e.g., bottles, jars, slides, covers, labels, drawing materials, and instruments—can usually be purchased from the Supply Department.

The Chemical Room Staff makes up various histological and photographic solutions, and certain standardized reagents used by investigators and classes for ordinary biological and chemical work. Such solutions and reagents are described in "Formulae and Methods," III, 1936. The Chemical Room does not undertake special chemical work for individuals or classes nor the preparation of solutions, requiring unusual time or facilities. However, for the period from June 24 to September 1, the chemist will undertake to standardize a limited number of special solutions for individual investigators; and to determine the pH of a limited number of aqueous solutions. It is preferable to submit at least 10 ml. quantities of well-buffered solutions or 20 ml. quantities of unbuffered solutions, although determinations will be made on less solution, if necessary. The samples should be placed in clean, stoppered containers which are labeled with the name of the investigator, the room number, the approximate pH if known, and a description of the contents, if of a hazardous nature—cyanide, for instance. The samples should be left at the Chemical Room before noon. The results may be obtained at the Chemical Room the following day. The number of determinations for any one investigator is necessarily restricted. Investigators expecting to use such solutions or standardized reagents after September 1 are requested to notify the Chemical Room, if possible, before August 24.

Special Supplies:

Dry ice may be obtained at cost in limited amounts through the Chemical Room. For dry ice, rare chemicals, etc., ample advance notice of at least three days is required. In the case of expensive reagents such as osmic acid, gold chloride, platinum chloride, special organic compounds and dyes or stains the Laboratory makes a charge at current prices for all above a reasonable amount used by an investigator. This rule may apply also to any other materials which are *scarce, difficult to obtain, not likely to be required by other investigators, or are requested in unusual quantities.*

Draughting supplies, portable microscope accessories, dissecting equipment, surgical instruments including syringes, needles, etc., are not available for loan but may be purchased through the Supply Department. Easily transported apparatus such as stop-watches, cameras, and devices of special application should be provided by each investigator (before departure for Woods Hole) unless he is assured that they can be furnished by the Laboratory.

Window Service, 1941:

June 30 to August 30 — 8:30-12:00 a.m./1:30-4:30 p.m. Saturdays—8:30 to 12:00 a.m.

Holidays excepted.

September 2 to 27 — 11:00-12:00 a.m./3:30-4:30 p.m.

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris I. Gorokhoff and Judy Woodring.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879, and re-entered, July 23, 1938.

SYMPOSIUM ON DEVELOPMENT AND GROWTH

The following is the program of the Symposium of the Society for the Study of Development and Growth to be held at Dartmouth College, Hanover, N. H., from July 7 to 11.

General Topic: **Patterns.**

Monday, July 7th

Morning Session: **Francis O. Schmitt** (Washington University, St. Louis.) "Patterns of Protein Molecules." Configurations, orientations, and properties of protein and conjagated protein components in the ultrastructure of cells and tissues. Supplementary data from mono- and multifilms and crystalline protein.

Afternoon session: **Vance Tartar** (University of Vermont). "Intracellular Patterns." Facts and principles concerning patterns exhibited in the morphogenesis and regeneration of ciliate protozoa.

Tuesday, July 8th

Morning session: **Kenneth B. Raper** (U. S. Dept. of Agriculture). "Patterns of Primitive Cell Collectives." Developmental patterns in simple slime moulds.

Afternoon session: **A. F. Blakeslee** (Carnegie Institution, Cold Spring Harbor). "Growth Patterns in Plants."

Wednesday, July 9th

Morning session: **N. J. Berrill** (McGill University). "Growth patterns in Lower Animals." Spatial and temporal patterns in colonial organisms.

No session in the afternoon.

Thursday, July 10th

Morning session: **A. H. Hersh** (Western Reserve University). "Heterogonic Growth." The ontogenetic and phylogenetic significance of differential rates of growth.

Afternoon session: **L. C. Dunn** (Columbia University). "Abnormal Growth Patterns, with Special Reference to Genetically Determined Deviations in Early Development."

Friday, July 11th

Morning session: **Paul Weiss** (University of Chicago). "Nerve Patterns." Analysis of the factors controlling the development of the nervous system as an example of complex pattern formation.

Afternoon session: Assignment not yet decided.

THE CHILDREN'S SCIENCE SCHOOL

The Children's Science School opened Monday, with a registration of 57 children. More have been registered during the week. The registration was followed by a showing of lantern slides by Mr. Lower, which was greatly enjoyed by the children.

The teachers this summer are Miss Helen Smith, who is also director, Mr. Reginald MacHaffie, and Mr. and Mrs. George G. Lower, all of whom were here last year.

At the opening meeting Monday afternoon, the teaching staff gave most interesting outlines of the courses they are giving this year. In Miss Smith's marine ecology course, the specimens are collected on frequent field trips and identified and preserved at the school. The biology course is also presented by Miss Smith. It consists of an introduction to the structures and functions of animals, and to some of the more important biological principles. Lectures are supplemented by experiments and dissections of interesting forms.

Mr. MacHaffie is hoping to do quite a bit of culturing of insects in his entomology course, with ant colonies, bees, etc. For the junior laboratory, he has several problems to work out, on which little research has been done. Genetics and embryology are particularly emphasized.

Mr. Lower is to have his very original system of charts, with teams, tests in various subjects, and awards with special credit given for collections. These are for both the Water Life and Nature Study classes, and he is also thinking of adding a bit on weather, tides and navigation to the more advanced class, explaining the buoys, fog signals, etc.

Mrs. Lower in her Introduction to Nature Study initiates the beginners in observation, collecting, classification, etc., and says that, with a little training, the youngest children become keen observers and make very creditable collections. She plans to instruct them early in the class on aquaria and keeping sea life at home.

These previews of the courses make it appear that the Science School has a particularly stimulating season ahead. —*Mrs. S. McMurtrie.*

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
July 5	12:59	1:16
July 6	1:56	2:12
July 7	2:54	3:08
July 8	3:48	4:00
July 9	4:37	4:53
July 10	5:28	5:40
July 11	6:13	6:30

ITEMS OF INTEREST

DR. EUGENE F. DUBOIS, professor of medicine at the Cornell University College of Medicine, has been appointed professor of physiology and head of the department of biochemistry and physiology at the College. He succeeds Professor Detlev W. Bronk, who returns to his post as director of the Johnson Foundation for Medical Physics at the University of Pennsylvania.

DR. DONALD P. COSTELLO, assistant professor of zoology at the University of North Carolina, will be on leave of absence next year. He has been appointed a Rockefeller Foundation fellow and will work at the School of Biology at Stanford University.

The Carnegie Corporation of New York has granted to Dr. L. J. Milne, associate professor of biology at Randolph-Macon Woman's College, about $900 with which to obtain special equipment for biophotography. This summer at Woods Hole he is collaborating with Dr. C. L. Parmenter in presenting cell division of salamander epidermis as animated diagrams on motion picture film.

DR. T. K. RUEBUSH is leaving for Washington, D. C., where he has been called to active duty in the Medical Corps of the U. S. Navy.

Among the persons who attended the Symposium on Genes and Chromosomes at the Biological Laboratory at Cold Spring Harbor and who will spend the summer at Woods Hole are: Dr. and Mrs. P. W. Whiting, Dr. C. W. Metz, Dr. Harold H. Plough, Dr. R. Ruggles Gates and Dr. Morris Harnly.

Notes from the Bureau of Fisheries

MR. ROBERT A. GOFFIN and his staff had planted 1,293,000 mackerel fry at the end of last week. The mackerel were stripped at the traps and the eggs fertilized and brought to the Hatchery. The Bureau of Fisheries carries out this conservation measure each season.

A new attraction at the Fisheries pool is the blue shark, approximately six feet long, which was taken in a fish trap in Buzzards Bay off Penzance Point. It is unusual for this shark to come so far inland. In addition, there are two seals and a few dogfish in the pool.

The registration for the month of June at the Bureau of Fisheries was 3,401. From July 1, 1940 to June 30, 1941, 25,099 persons wrote their names in the visitors' book. In general, Mr. Goffin finds that about one in four persons who come to the aquarium register there.

At the Durham meeting of the American Association for the Advancement of Science, the program of the Section on Medical Sciences included a paper on properties of skeletal muscle fibers by Dr. F. J. M. Sichel, assistant professor of physiology at the University of Vermont College of Medicine. Mrs. Sichel went to New Hampshire to read the paper for him owing to his conflicting engagement with the physiology course.

DR. ALBERT NAVEZ, of the Milton Academy, presented a paper on "Biology, the Science of Life (Plea for the Experimental Method)," on June 25 before the National Association of Biology Teachers at the Durham meetings.

The Spencer Lens Company is holding its exhibit at the Canteen Building until Friday, June 11.

The program for the weekly phonograph record concert at the M.B.L. Club next Monday is as follows: Beethoven, "Overture to Leonore, No. 3"; Beethoven, "Symphony No. 5"; intermission; Brahms, "Symphony No. 8."

DR. T. K. RUEBUSH, the secretary-treasurer of the Tennis Club, announces that a tennis tournament will be scheduled this summer if enough people show interest and apply to take part in it. Anyone who wishes to enter such a tournament should see Dr. Ruebush, Dr. D. E. Lancefield, or Albert Stunkard.

The Woods Hole Choral Club opened its season with a very successful rehearsal Tuesday night at the Canteen Building of the Bureau of Fisheries. A group of over thirty persons connected with the Laboratory was present, and practiced four of the songs which will be sung at the annual concert late in August. The next rehearsal of the Club will be held on Tuesday.

ADDITIONAL INVESTIGATORS

Clark, Eleanor L. assoc. anat. Pennsylvania Med. Br 117.
Erlanger, Margaret instr. West Virginia. Br 312.
Finkel, A. J. grad. asst. zool. Chicago. Br 322.
Gelback, Elizabeth L. asst. proto. Yale. Br 323.
Hamilton, H. L. res. asst. emb. Hopkins. Br 324.
Harnly, M. H. assoc. prof. biol. New York. Br 344.
Horn, Annabelle grad. asst. zool. Pittsburgh. Rock 7.
Keefe, E. L. res. asst. zool. Washington (St. Louis). Br. 217-j.
Klotz, J. W. grad. zool. Pittsburgh. Rock 7.
Michaelis, L. mem. Rockefeller Inst. (New York). Br 207.
Mullins, L. J. asst. phys. Rochester. Br 322.
Netsky, M. Pennsylvania Med. Br 205.
Sandow, A. asst. prof. biol. New York. Br 344.
Spratt, N. T., Jr. res. asst. emb. Hopkins. Br 324.
Trager, W. assoc. Rockefeller Inst. (Princeton). Br 208.
Weaver, Margaret A. grad. zool. Texas. Br 312.

PHYSIOLOGY CLASS NOTES

Our second two weeks' period of work is now in full swing. The tables have been turned and now we are showing Dr. Fisher how to run the Warburg apparatus. It does our hearts good these days to see him taking down manometer readings, and cleaning vessels. We hope his results are better than ours were.

The home-run king, Dr. Parpart, began the lectures this week, talking on permeability, and the composition and structure of cell membranes. Thursday Dr. S. C. Brooks lectured to the class, the subject being "Some Problems in Permeability." Our first guest lecturer was Dr. L. V. Heilbrunn, who spoke last Saturday on the theory of muscular contraction with special reference to the rôle of ions. It was he who quoted the great physiologist and chemist, Loeb, as saying that if you don't have brains, use apparatus. We use apparatus.

There are now four sections running parallel under Drs. Parpart, Kempton and Ballentine. One group of Dr. Parpart's students is studying permeability using dogfish red cells and arbacia. Another group is determining the total lipid and cholesterol contents of dogfish ghosts. Here again is something we can't see. A third group is studying the hemolytic effects of detergents, and a fourth is using cleavage curves of arbacia eggs to determine permeability.

Part of Dr. Kempton's class may be found in the basement micro-manipulating, while the others are upstairs learning why the kidney. Dr. Ballentine's students are determining the intercellular proteases during development of arbacia, and the distribution of dipeptidase in arbacia eggs. This is running along smoothly because at least one member of the group has worked on eggs before,—hen's eggs. To quote this particular member, "Arbacia eggs are very tiny."

In the blood gas studies group, with the aid of Drs. Parpart and Ballentine, oxygen dissociation curves of limulus hemocyanin, spectrum absorption curves in oxygenated hemocyanin, and manometric methods are being emphasized.

"Come on Physie, keep 'em busy!" yelled the sideline physiologists, as they cheered their team on to victory. It all happened Monday evening when we met our friends the embryologists out upon the baseball field. We are happy to say that we came out on top, with a score of 18 to 14, but not without a few anxious moments. It was a good game, from both sides, and it was only through the able pitching of Bob Harrison, who also hit a home-run and brought three others in; the whole-hearted cooperation of our class and the faculty representative, Dr. Kempton; and the brains back of the team (those of manager Bill Keezer) that we accomplished the deed.

—And then came Mr. Trinkaus up to bat for the embryologists. Strike one! Strike two!! Strike three!!!—Just another sad story of *The Third Out,* or *With the Bases Full and* (all due apologies to the poem of the same name hanging in Dr. Fisher's office) *Nobody Ohm.*

The embryologists join us in thanking Ted for doing such a good job as umpire.

We must not forget the game played last Friday afternoon—faculty et all. Maybe it was the practice they gave us that helped us win on Monday. The highlights of the game were Dr. Parpart's home run and Dr. Fisher's striking out.

We are looking forward with a great deal of pleasure to our class picnic which has been planned for next Tuesday. Arrangements are being made by the committee, Bob Harrison and the Black Phantom (his car). —*J. E. H.*

BOTANY CLASS NOTES

Sometimes I have to wonder why
The artist's life I did deny,
For plants, I find, are not elating
And skunky Chara's most nauseating.

True as this is in lab, collecting is another story and a much more pleasant one: the Cuttyhunk expedition was a perfect vacation trip. A picturesque little fishing village clinging to the hillside, the stone-walled road snaking through it and up into nowwhere, the Coast Guard look-out box on the highest point, the flashing twin lakes (Chara gold mines), miniature orchids in the deep sphagnum gullies, a storm-worn monument to Gosnold (probably rich in aerial algae), the long strand of bright, white sand, intensely blue water, lazy gulls—all enough to convince one of the authenticity of a National Geographic illustration. But romanticism went by the board when nineteen botanists plunged into the waist-deep muck of Euglena Pool and waded through the waters of Sheep Pond. That night in lab we learned that this disagreeable muck is an algologist's paradise, having species brought from foreign parts by none other than the lowly sheep that bathe there on arrival from the mainland.

Class field trips, however, are not the only exercise we get. "I'll never in all my life forget the sight of Monti standing on the dock with six girls around him, wildly gesticulating and almost hidden by the fog!" reminisced Connie Stanton in recalling the Monti Expedition to Nonamesset Island. If you happened to see a gentleman striding down the Main Street last Saturday afternoon, followed by six somewhat feminine-looking

creatures, it was not a sultan with his harem, but just an innocent lad leading a bevy of blue-jeaned botanists to the Oceanographic Pier. The former algology student had in some way procured two skiffs for the trip. Being a true celibate at heart, Monti allotted one to Bob Thorne and very generously sent him out to sea with only two of the fair sex for crew, setting out with the remaining half dozen himself. The greatest fun occurred when the waves—choppy and very wind-blown—proved too much for the overloaded rowboat and forced the hero to sacrifice his adventurous plans for the delights of retaining his harem. Bob, meanwhile, faring better with his smaller group, was able to master the ocean, although the defeated seven finally persuaded him to quit his efforts in favor of a hike to Nobska, via the more tranquil terra firma.

Such ideas, especially those of hiking, are typical of the Botany Class. As if the all-day work, five and a half times a week, were insufficient, certain fanatics in the course find it necessary to devote part of the Sabbath to quenching their thirst for knowledge. So that bright and early—about 8:30 A. M.—on beautiful foggy Sundays, Bob Thorne may be seen leading his devotees out to gather the fair posies of local habitats. Last Sunday, however, the presence of Charlie Abbot, welcomed by everyone, seemed to worry Mr. Thorne to such an extent that he retired within his anti-female shell and did a much more thorough job of collecting.

A collection of algae specimens is, of course, the ultimate tangible result of this class, but equally interesting tangents are continually adding to the less mountable, general knowledge that the botanists are also collecting. An extra bit was added last Thursday night when Bob Williams gave a very informative lecture about carbohydrate metabolism in the large brown algae. What with his illustrative graphs and his slightly shaky knowledge of the larger details of organic chemistry, the audience found the seminar far from dull. Tea, as usual, climaxed the program.

Evening teas, though, are hardly long-lived enough to stave off the starvation pangs in mid-afternoon. Several would-be-fortunate recipients of boxes from home—chiefly Elaine Katz—were completely submerged and left entirely bare of all food-stuffs when the hungry class descended upon them. Which all goes to prove something, one would suppose. At any rate, there's no doubt that the competitive spirit runs abnormally high at such times—particularly between Bob Muir and Sam Salvin who with all the grace of true gentlemen, inevitably consume the greatest amount of the booty. But then it's every man for himself, with the fastest one the winner. —*J. W.*

EMBRYOLOGY CLASS NOTES

A new idea was brought to our attention Tuesday morning by Dr. Goodrich in his talk on the patterns of chromatophores in goldfish. Apparently one can have one's fish monogrammed according to order by transplantations of scales.

The squirting squid was squarely squelched when Dr. Hamburger demonstrated "How Squids Squirt." Looking at the various sketches made by the class we were carried back to our childhood stories and Walt Disney's interpretation of Mother Goose. To become more scientific, the development of the squid eye was of interest because of its similarity to the mammalian eye, although its parts are not homologous.

Dr. Hamburger's lecture on embryonic induction where the history was traced and theories old and new presented was one of the high spots of the week.

Coelenterates were presented by Dr. Ballard, whose humorous comments on the life histories of various members of that Phylum were enthusiastically received. How nice to lead a double life as they do by metamorphosis!

Tunicates are our ancestors or so we were told. Apparently they are the other extreme of the phylum Chordata, to which *Homo sapiens* belongs.

The week ended with a lecture by Dr. Schotté (sans chapeau—he lost it) opening the work on echinoderms.

The high spots of the unscientific week were initiated by our first baseball game with the crew. We really didn't do so bad considering their previous experience. The embryologists work, the crew—well, maybe.

The Second Trinkaus has arrived and was promptly put to work collecting towels which Ted distributes. While we are on the subject—the First Trinkaus, together with an eminent instructor received a biological baptism early in the week when a canoe upset in front of the M.B.L., a very appropriate place. One watch, the only casualty, was given first aid—dehydration.

The dance on Saturday night was quieter than that of the preceding week. Embryology was well represented.

Our cultural life was taken care of by a concert at the M.B.L. Club which, added to the harmony by Jack, Tom and Dr. Ballard in the lab at night, greatly increased our musical appreciation.

As this goes to press we are feeling greatly encouraged. The softball score between classic rivals is swinging in our favor, as was shown in the game tonight with the people in the "back room." —*P. H. and E. K.*

The A. B. C. of Woods Hole for 1941

All Schedules Set to Daylight Saving Time — Bold Type Indicates P. M.

POST OFFICE

	Week Days	Sundays
Mail Arrives	8:00, 10:55, 3:45, 7:15	10:35
Mail Closes	6:30, 9:30, 5:00	5:00
Station Open	6:00 to 8:00	10:30 to 5:15
Window Service	7:30 to 6:00	.....

All mails should be deposited at least ten minutes before closing time to insure dispatch; registered letters should be deposited fifteen minutes before closing time.

TELEGRAPH OFFICE

Weekdays
8:00 to 9:00

Sundays
10:00 to 12:00
4:00 to 6:00

Holidays
9:00 to 11:00
4:00 to 6:00

LIBRARY HOURS

Mondays, Wednesdays and Saturdays

3:00 to 5:00
7:00 to 9:00

RELIGIOUS SERVICES

Church of the Messiah (Episcopal)
Sundays: 8:00 Holy Communion; 11:00 Morning Prayer (Choral Eucharist, first Sunday in the month).
Holy Days: 8:00, Holy Communion.

Methodist Episcopal Church
Morning Worship, 11:00. Church School, 10:00.

First Orthodox Congregational Church
Evening Service, 7:30.

St. Joseph's Roman Catholic Church
Mass: Sundays, 6:45, 9:30.
Weekdays, 7:00.

TRAIN SCHEDULE*

	Weekdays	Weekdays	Ex. Sat. & Sun.	Weekdays	Sundays	Sundays¶
Woods Hole	7:06	10:15	**12:55**	**5:45**	**6:00**	**7:55**
Boston	9:15	**12:35**	**2:52**	**7:57**	**8:10**	**9:55**

	Weekdays	Sundays	Saturdays¶	Weekdays	Ex. Sat. & Sun.	Weekdays
Boston	8:20	8:35	**12:25**	**1:05**	**4:00**	**5:00**
Woods Hole	10:45	10:45	**2:30**	**3:25**	**5:59**	**7:10**

*All trains stop at Falmouth. ¶Discontinued after August 31.

BOAT SCHEDULE*

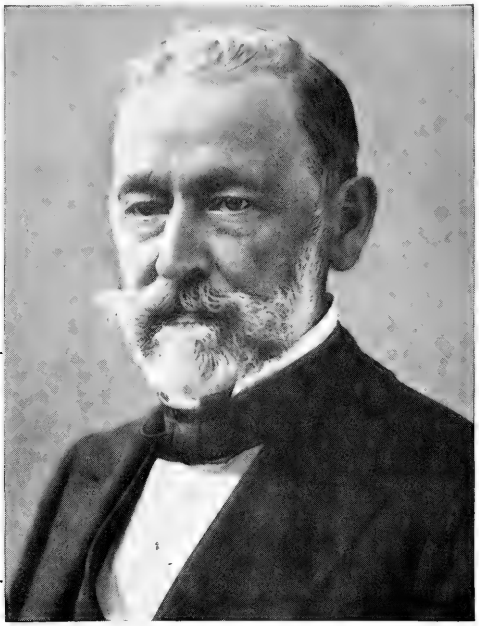
Leaves	Daily	Daily	Weekdays¶	Daily	Weekdays‡	Fri., Sat., Sun.£	Fridays¶
New Bedford	7:00	9:30	**2:00**	**2:30**	....	**7:30**	....
Woods Hole	8:30	10:50	**3:15**	**3:50**	**7:15**	**8:45**	**9:30**
Oak Bluffs	9:20	11:40	**4:00**	**4:50**	....	....	**10:15**
Vineyard Haven	....	....	**4:30**	....	**8:00**	**9:30**	....
Nantucket (due)	11:35	**2:00**	....	**7:00**	....	....	12:15

Leaves	Daily	Daily	Sundays¶§	Daily	Weekdays‡	Daily	Sundays§
Nantucket	....	6:45	**2:00**	**2:30**	....	**4:45**	....
Vineyard Haven	6:10	....	....	....	**6:00**	....	....
Oak Bluffs	....	9:00	**4:00**	**4:30**	....	**6:45**	**9:00**
Woods Hole	6:55	10:00	**5:00**	**5:30**	**6:45**	**7:30**	**9:45**
New B'df'd (due)	8:15	11:15	....	**6:45**	....	**8:45**	....

*Schedule effective to Sept. 6, incl.
¶Discontinued after Aug. 30.
‡Does not run Labor Day.
£Runs 15 min. later on Sunday, daily after August 31.
§Also runs Labor Day.

The Spencer Trunnion Microscope, such as made in 1847.

Looking forward



C. A. Spencer, the first American microscope maker, 1813-1881.

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See exhibit of complete Spencer equipment in Canteen Building, Bureau of Fisheries, June 30 to July 11.

THE COLLECTING NET

Vol. XVI, No. 3 SATURDAY, JULY 12, 1941 Annual Subscription, $2.00 Single Copies, 30 Cents.

THE SOURCE OF PANCREATIC JUICE BICARBONATE

DR. ERIC G. BALL

Associate Professor of Biochemistry, Harvard Medical School

Pancreatic juice that is rapidly secreted is in osmotic equilibrium with blood plasma but contains mainly sodium bicarbonate as its inorganic constituent. This means that pancreatic juice may contain five to six times the quantity of bicarbonate ion that is found in blood plasma. What then is the source of this juice bicarbonate? Two possible sources exist. One is the bicarbonate of the blood flowing through the gland. The other is the CO_2 produced by the metabolic processes of the gland itself. At the start of this investigation we were inclined to the view that a considerable part of the bicarbonate of the juice was derived from the metabolic CO_2 of the gland itself, for two reasons. First, because the amount of bicarbonate in the juice varies with the rate of juice secretion. The more rapid the rate of secretion the higher the bicarbonate content. Such a relation can be interpreted to mean that (*Continued on page* 55)

M. B. L. Calendar

TUESDAY, July 15, 8:00 P. M.

Seminar: Dr. L. B. Clark: "A Suggested Mechanism by which the Moon Influences Reproduction in the Atlantic Palolo Worm."

Dr. Paul S. Galtsoff: "Accumulation of Manganese and the Sexual Cycle in Ostrea virginica."

Dr. R. M. Cable and Dr. A. V. Hunninen: "Studies in the Life History of Siphodera, a Trematode Parasite of the Toadfish."

Dr. H. W. Stunkard: "Pathology and Immunity to Infection by Heterophyd Trematodes."

FRIDAY, July 18, 8:00 P. M.

Lecture: Dr. Eric Ponder: "Red Cell Structure in the Light of Shape Transformation."

CURRENT APPROACHES TO THE PLANT HORMONE PROBLEM

DR. GEORGE S. AVERY, JR.

Professor of Botany, Connecticut College

By definition, whether in animals or plants, hormones are chemical substances normally produced in the cells of some part of an organism, and transported to other parts where in one way or another they regulate growth and metabolism. Of the many factors which regulate growth, hormones constitute only one; their importance to the development of living organisms lies first in the fact that they are produced internally (they are not ordinarily taken in from the environment, for example, as plants take in minerals, etc., from the soil), and second, that they exercise their regulatory effects when present in minute amounts.

Plants do not possess secretory glands, like those of animals, but the embryonic regions such as growing points of roots, stems, etc., where new protoplasm is constantly being synthesized, are the centers of hormone production.

One of the better understood but not often dis-

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AERIAL VIEW OF NONAMESSET, PENZANCE POINT, AND THE ISLANDS BETWEEN

The south end of Nonamesset Island appears in the foreground with Sheep Pen Cove on the right. The islands reading from the foreground to the background are: Pine, Devil's Foot and Ram Islands. The ribbon of land in the background is Penzance Point; the tip of Dr. Warbasse's estate can be seen at the extreme left.

cussed hormones in higher plants is vitamin B_1, thiamin. It is normally produced above ground in the green tissues of the plant, and is transported to the roots. Root growth, in many species at least, cannot go on without it. If roots are excised from the parent plant, and grown in culture, thiamin has to be supplied in the nutrient medium. Thus, in *in vitro* experiments it is no longer a hormone (by definition), but is more nearly akin to the growth factors, or accessory substances, of microorganisms. I mention this only to show that definitions of the past are breaking down as we learn more about the physiologically active substances produced by living organisms. That hormones are "activators" and "correlating substances" of one sort or another, all will agree. Let us leave it at that.

Before current approaches to the problem are mentioned, those of you who are unfamiliar with the field will want a little more information, particularly about the more commonly discussed *auxins*. Only three auxins have been isolated from higher plants: auxins *a* and *b* of Kogl, and 3-indoleacetic acid. A considerable number have been synthesized. In spite of the chemical differences in the numerous substances known, they all bring about the same general non-specific physiological effects, *e.g.* they promote growth in length of stems, and retard growth in length of roots.

The history of the discovery of the auxins has been treated in detail in "Growth Hormones in Plants" by Boysen Jensen *et al,* and in "Phytohormones" by Went and Thimann. It is an interesting record of scientific discovery in which the chief object of investigation has been the young shoot (coleoptile) of the grass seedling. The study of the response of the coleoptile to light and gravity has led, over a period of fifty years, to the discovery and chemical identification of growth hormones in plants.

Methods of assay. The advance of quantitative biology, whatever the special field, usually rests on the development of methods for measuring. For hormones, chemical methods would be the ideal, but thus far no chemical test has been devised which is sufficiently sensitive to detect any of the known plant hormones in the low concentrations in which they naturally occur. Thus, as in so many instances in animal physiology, a living test organism is essential. Went gave us the *Avena* (oats) coleoptile test in 1928. It has undergone a number of modifications since, but apparently is still the most dependable and most quantitative test devised to date. In our laboratory we use the "deseeded" modification suggested by Skoog (details can be omitted here). It still requires a constant temperature constant humidity darkroom. A test chamber has been developed which simplifies greatly the equipment needed for making hormone tests, *i.e.,* no humidity control is necessary. The "deseeded" *Avena* test method makes possible quantitative estimates of indoleacetic acid in concentrations as low as 5 to 10 micrograms per liter of solution, and with such synthetic growth substances as alpha naphthaleneacetic and indolebutyric acids it will give good quantitative determinations on concentrations down to 25 to 50 micrograms per liter.

As for the potency of plant hormones: if it were physically possible to place a million *Avena* seedlings side by side, with their coleoptiles actually touching one another, they would extend for a distance of about one mile. And if it were possible to cut the tips off all these coleoptiles and place tiny agar blocks containing indoleacetic acid on one side of coleoptile stump, (as in the Went test) one milligram of indoleacetic acid would be enough to cause the mile of coleoptiles to grow on the side to which the hormone was applied, and the final result would be a ten degree curvature of the coleoptiles (from the vertical position).

A considerable number of test methods other than with coleoptiles have been devised, at least one of which will detect much smaller amounts of auxin than those just indicated; the difficulty is that it is not very quantitative. Green tissue test objects are also in use, but in general they are not very sensitive.

The search for better methods of assay still goes on, and in the past year two new ones have been developed. Parker-Rhodes proposes a test for plant hormones based on osmotic pressure changes in root hairs of wheat. This is a wide departure from previous methods, practically all of which depend upon tissue responses and growth curvatures of one sort or another as the end result. The other new method is that of Turfit, who reports, contrary to the earlier studies of others, that cell division in yeast is affected by both naturally occurring and synthetic auxins; thus he measures increased CO_2 output, or after a few hours counts cell numbers in yeast cultures . . . the increase in cell number being very roughly proportional to concentration of hormone present in the culture medium. It is too soon to evaluate these methods fairly, though it is clear that they are not free from difficulties. Their adop-

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tion by other workers will be the index of their success.

Units. A standard unit of one sort or another is as necessary as a good and universally used assay method, that is, if workers are to be able satisfactorily to compare their results. Some progress has been made along this line. Overbeek has proposed that hormone content of a tissue be expressed in terms of a compound of known physiological activity, and has selected indoleacetic acid as that compound. Thus, after assay, one may say that the hormone content of a tissue is so many gamma (or microgram) equivalents of indoleacetic acid per kilogram of tissue.

There is one difficulty in the otherwise ideal proposal Overbeek has made. He originally supposed that "gamma equivalents" would be independent of the test method used, but it has since been shown that different test methods give different results with the same hormone extract, *i.e.*, two different modifications of Went's method give widely different assays when hormone content is expressed in terms of gamma equivalents of indoleacetic acid. Thus, for the present at least, standardization of units is not possible.

Getting hormones out of tissue. The "diffusion" method was for a long time the only one used. This procedure consists of standing a piece of plant tissue on a small rectangular plate of agar for a standard length of time; the agar is then cut into small blocks, and applied to decapitated coleoptiles . . . the *Avena* test. This presumably gives an index of the hormone concentration in the tissue being tested, but does not give any quantitative value for the hormone *content* of tissue. The necessity for quantitative extraction is clear, if we are ultimately to have an understanding of the role of hormones in growth.

Here are some of the more recent steps in the direction of better extraction methods: Boysen Jensen five years ago proposed an ether extraction method, whereby the tissue being extracted is placed in freshly distilled ether which has been freed from peroxides. The tissue is allowed to stand overnight in two changes of ether. The following day the ether extract of hormone is taken down to dryness, and the residue is taken up in a small amount of 1.5% agar. *Avena* assay of the agar-hormone mixture follows.

It has since been found that if fresh ether is added weekly to a sample of tissue from which the hormone is being extracted, that hormone will continue to be liberated from the tissue for a period of several months, thus suggesting the presence of an ether insoluble compound which is slowly hydrolyzed into auxin. Thimann and Skoog (and others) have struggled with this problem of extracting hormone from green tissues, and have recently reported on a number of methods. Some of them gave good yields, but with only one tissue were the results to the satisfaction of the authors.

In our laboratory we have sought and found a method for total extraction of hormone from non-green tissues such as the storage tissues of seeds, etc. It give reproducible results and extraction takes only a few minutes. It involves heating an aqueous suspension of the tissue at 100 to 120 degrees C. for fifteen minutes, at a pH of 9 to 10. After heating, the suspension is centrifuged and the pH of the clear extract is adjusted to approximately 6; agar blocks are prepared from this aqueous extract of the hormone, and assayed by the *Avena* method. Corn endosperm extracted by this method gives yields as high as the equivalent of 150 milligrams of indoleacetic acid per kilogram of tissue (content varies according to variety). This is a higher yield than has yet been reported, and is due to the conversion of a "precursor" compound into auxin. The auxin present in such great quantities in maize endosperm has been shown to be indoleacetic acid, and the chemical identity of the precursor is just about to be established.

These discoveries of the past few months mark an important advance in the extraction and identification of naturally occurring physiologically active substances. Less recent, but equally important, are the isolation and identification of wound and leaf growth hormones at the California Institute of Technology. Auxins *a* and *b* are now an old story and may turn out to be less important than originally thought. The chief point is that the plant hormone family is increasing in number, and in addition to those growth promoting hormones already mentioned, it seems likely that one or more substances specifically concerned with differentiation may soon be isolated and identified, *e.g.*, flower and sex differenting substances.

Hormones and normal growth. Methods of extraction and assay would be of little value if they could not be applied to problems of growth and differentiation. What evidence is there for auxins being agents which exercise developmental control in organisms? Here, in brief, are the results of a few studies: It has been shown in certain leaves that the regions of greatest growth intensity are also the regions of highest auxin concentration; that plant shape and rate of development of plant organs may be correlated with auxin concentration; that the "cambial stimulus" in trees (initiation of lateral growth in the spring) is related to auxin concentration; that dwarfing in maize is related to destruction of auxin; that "lazy" maize, which as its name implies falls over and grows on the ground, gets its lazy habit at least in part from mal-distribution and mal-transport of auxin in its tissues. Other examples might be cited, but this is a quiet sector on the hormone

front just now. Better extraction methods must be developed before too much faith is placed in studies on growth in relation to hormone content of tissue.

Synthetic plant growth substances. For all practical purposes, we might as well call these substances "hormones" also. Most of them were first reported by Zimmerman and Wilcoxon in 1935. A few of them (indoleacetic acid is now in the "naturally occurring" group for higher plants), in the approximate order of their potency, are alpha naphthaleneacetic acid, indolebutyric acid, indolepropionic acid, phenylacetic acid, etc. A newcomer, beta naphthoxyacetic acid, is bidding for an important place; in addition to bringing about responses in the usual curvature and other tests, it will also induce form changes if sprayed on plants in high concentrations (or if the plants are watered with it). Except for the preliminary work of Pearse, this is the first compound of a hormone nature with which it has been possible to demonstrate morphogenetic effects in a living intact plant; needless to say, this marks an important advance.

Horticultural applications. The use of synthetic hormones in the rooting of cuttings of higher plants is probably the best known of all the new practical applications, and "rooting compounds" are on sale in almost all seed stores. When sprayed on, or otherwise applied to flowers, under the proper circumstances, seedless fruits are developed . . . and such seedless tomatoes, I am told, are in commercial production. Eggplant, squash, peppers, cherries and even ornamental holly berries are now on the potential "seedless" list, but worse, watermelon tradition is going to be changed . . . for it is possible to have seedless watermelons too.

Perhaps the most important horticultural use thus far is spraying these substances on apples etc. to prevent pre-harvest drop of fruit. The manner in which hormones prevent abscission is not yet understood, but that they are abscission-controllers, there is no doubt.

Hormones in relation to abnormal growth. Not new, but of increasing significance, is the work of Kraus *et al* at the University of Chicago. He and his coworkers have applied high concentrations of synthetic hormones to bean, and other species, and studied the tissue changes which followed. Ultimately the overgrowths produced develop into good-sized galls. They are not "cancers", but are perhaps as cancer-like as anything which plants can produce. Chemical analysis of the treated tissues indicate that the applied hormones are responsible for mobilizing nitrogen, carbohydrates, etc., at the place of application. This is very suggestive. Is it possible that animal cancers secrete mobilizing substances, *i.e.,* that once started they continuously secrete such substances, thus perpetuate themselves?

Treatment of sunflower and rose stems with certain of the animal carcinogens has failed thus far to bring about the response just noted for plant hormones, but the problem needs re-examination with younger test material which might have greater capacity to respond. Of course it may be that the water insolubility of these compounds makes it impossible for plant cells to take them in in quantities sufficient to bring about a response.

The spontaneous tumors occurring in the hybrid of *Nicotiana glauca* × *N. langsdorfii* deserve attention in passing.

The role of hormones in the production of plant tumors is as yet but little understood. It is important for a general understanding of growth that we go further into the problem. Alert students will find a fertile field.

Possible roles of hormones in plant growth. Mention of mobilization effects attributable to hormones has just been made, but it seems unlikely that the primary effect of hormones would be on mobilization. Critical studies of hormone influence on metabolic rates must be made, and extended studies on hormone-enzyme relationships should prove very fruitful; on this latter point there are some interesting suggestions already. It has been known for some time that auxin is present in higher concentrations in the tip than elsewhere in the *Avena* coleoptile, and recent studies of peptidase distribution in the coleoptile show that peptidase activity is also higher at the tip. Is auxin acting as an enzyme activator, as has been suggested? Further promising work along this line has been done by Commoner and Thimann, who give limited evidence of auxin activation of certain events in the respiratory chain.

The fact that different auxins can bring about the same general physiological responses in plants (whatever specificity there is, residing in the species) is analagous to the coenzyme-enzyme relationship, where a single substance is known to act as a coenzyme for a number of enzymes, and the specificity of the combination resides in the enzyme.

Although not often discussed along with the auxins, thiamin is the one plant hormone of which at least one role is understood. It has been shown in *in vitro* experiments to act as a cocarboxylase. There is nothing in the chemical structure of the various auxins to indicate whether they, like thiamin, may be acting as coenzymes, but the preliminary evidence favors the view that they act as enzyme activators, or components of enzyme systems.

The really important problems remain to be solved. We know little about the production of such physiologically active substances by organ-

isms, or how to extract and identify them. And we need much more information on the destruction of such substances in living tissues, more on inhibiting substances of one sort or another, and most of all, we need to know what role they play in growth and development.

Growth factors for microorganisms. Now just a little material to correlate the foregoing with the studies being made on the growth factors or accessory substances for microorganisms.

Certain microorganisms do not possess the ability to synthesize optimal amounts of these growth factors, and it is by supplying such deficient substances through the culture medium that we have learned about the necessity for them. Some of the substances in question are known to act as coenzymes or parts of coenzyme systems (thiamin, nicotinic acid, etc.), and it appears as if their roles in the growth of microorganisms were analagous to those of auxins and other hormones in higher plants.

From the microorganism work have come some interesting results, from the viewpoint of helping us to understand symbiosis and parasitism. Kögl and Fries, for example, grew two totally unrelated fungi in the same culture. One produced biotin, needed by the other for satisfactory growth while the other produced thiamin, needed by the first for good growth; the result was symbiosis *in vitro*.

A suggestion for the future. How may such information spur new research approaches on the relation of physiologically active substances to growth and development? Lichens may well be the plants we ought to study in this connection. They are compound organisms which exhibit symbiosis. One of the component organisms, the alga, synthesizes food which it delivers to the fungus component, while the latter supplies minerals and water to the alga. This relationship has been explained in the past as a perfectly straightforward nutrient partnership. The situation now seems less simple: it is undoubtedly more than a nutritive bond which holds an alga and fungus together, and produces an organism with new characteristics, an organism which is more than the sum of its parts. So stable is this relationship that the resulting plant bodies require generic and specific names. Nowhere else in the plant kingdom, so far as I know, do two completely different organisms live in such intimate relationship and produce specific new physical forms. There are no genes for lichens, and nutrition offers only limited possibilities of explaining the mode of their existence . . . it contributes nothing to an explanation of the new physical form assumed. Thus it appears that each component is secreting one or more substances of a "growth factor" nature which are influential in determining the form of the dual organism. If a lichen were a single organism we would say that its form is dependent upon its genetic constitution, its genes. There is a good likelihood, it seems to me, that growth factor studies on the lowly lichen may well provide a means of getting at the problem of the chemistry of the gene.

(This article is based upon a lecture delivered at the Marine Biological Laboratory on July 3.)

THE PERMEABILITY AND THE LIPID CONTENT OF THE ERYTHROCYTES IN EXPERIMENTAL ANEMIA

Dr. Arthur J. Dziemian

Department of Physiology, School of Medicine, University of Pennsylvania

Bodansky, (*J. Biol. Chem.*, 63:239, 1935) reported, a number of years ago, that administration of phenylhydrazine and allied compounds to animals brought about changes in the lipid content of the red blood cells. This paper describes experiments on the effect of phenylhydrazine on the permeability of the erythrocyte and its lipid content.

Albino rabbits were used in all these experiments and phenylhydrazine hydrochloride solution was injected subcutaneously. In five animals changes in the red cell counts, hematocrits, reticulocyte percentages, erythrocyte diameters, and permeability of the red cells to glycerol, diethylene glycol, ammonium propionate and ammonium salicylate were followed during the onset of and recovery from phenylhydrazine anemia after a single dose of the drug.

The number of red cells decreased rapidly, so that about the sixth day after treatment, the count was only about one quarter of the original value. After the sixth day the number of erythrocytes slowly returned toward normal. Hematocrit changes followed the variation in red cell count rather closely, with differences due to changes in the size of the erythrocytes.

About the second day after treatment, the reticulocyte percentage increased rapidly, new cells coming in to replace the original phenylhydrazine poisoned red cells. The percentage rose to between 30 and 40% of the total number of red cells on the seventh day, and then dropped back to the normal value of below 1% by the 16th day after administration of phenylhydrazine.

The mean diameter of the erythrocytes decreased for the first few days of the experiment,

but as the new reticulocytes entered the circulation, the average size of the cells was considerably increased above normal. The reticulocytes are of large size, some being eleven micra in diameter, as compared with a normal average of 6.8 micra. After the 9th day the new cells began to assume normal size slowly. Comparisons of frequency distribution curves of the diameters of the cells on various days after treatment showed that by the 8th day practically all the original shrunken cells were gone, indicating an almost complete new population of erythrocytes.

Permeability of the red cells was measured by rates of hemolysis of the erythrocyte in buffered solutions (pH 7.4) of the penetrating substances at 20 degrees C. The time to 50% hemolysis was taken as a measure of permeability.

Phenylhydrazine HCl *in vitro* has no effect upon the permeability of the erythrocyte, nor does it have any hemolytic effect, even after the cells are in contact with it for several days at 38 degrees C. The theory of its action *in vivo* is that the hemoglobin of the cell is changed in part to free hemin and reduced globin, which catalyzes the change of the rest of the hemoglobin to methemoglobin. Erythrocytes containing methemoglobin are supposed to be more readily attacked by the reticulo-endothelial system.

The rate of penetration of glycerol into the erythrocyte of the treated rabbits increased greatly, being fastest about the 8th day after injection of phenylhydrazine. It then became slower, taking about 80 days to reach the original value. In some cases the erythrocytes of the treated animals were from 20 to 29 times as permeable as the erythrocytes of the same rabbits before administration of the drug. Diethylene glycol penetration followed much the same course as did glycerol.

For the first couple of days after treatment, the erythrocytes showed an increase in permeability to ammonium propionate and salicylate, which paralleled the decrease in cell size. But as new cells came into the blood, the time to 50% hemolysis increased above the original value, and remained above normal for several weeks. Whereas the young cells of the rabbit were more permeable to glycerol and diethylene glycol than were the normal erythrocytes, they were less permeable to ammonium salts of organic acids, the so-called lipid-soluble substances.

The erythrocytes of eight normal albino rabbits were analyzed for total lipid, cholesterol, phospholipid, and neutral fats by gasometric methods. The permeability of these cells were also ascertained. The animals were then injected with phenylhydrazine hydrochloride, and the determinations were repeated. Blood was taken at various times after treatment, in some cases when the blood contained a high percentage of reticulocytes and in others after the rate of inflow of immature cells had decreased. In all cases the amount of total lipid per erythrocyte increased, but so did the area of the erythrocyte. When the total lipid, cholesterol, phospholipid and neutral fat contents were calculated per unit area of cell surface, there was no regularity, the distribution seemed random and not outside normal variation.

The results on permeability showed that the resistance of the treated red cell decreased in every case studied to glycerol and diethylene glycol. The figures for 50% hemolysis of the erythrocytes of the treated animals in the ammonium salt solutions indicated that they were taken at different times during the onset of and recovery from the anemia. In some the penetration was faster, the blood having been taken shortly after treatment, and in others, slower, having been drawn about ten to twelve days after injection.

No correlation seems possible with these results. They indicate that it is not the amount of lipid in the red cell surface that controls the permeability of the erythrocyte to the penetrating substances studied.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 8.)

SYMPOSIA AT THE UNIVERSITY OF CHICAGO

In connection with the fiftieth anniversary celebration of the University of Chicago at the end of September, and a special meeting of the American Association for the Advancement of Science convening there, symposia will be held in the various fields of human learning.

The symposia scheduled in the field of biology are:

"Growth and Differentiation in Plants"—**Chairman**, Ezra J. Kraus. **Speakers:** Charles E. Allen, University of Wisconsin; Edmund W. Sinnott, Yale University; John W. Mitchell, U. S. Department of Agriculture; John M. Beal, University of Chicago.

"Levels of Integration in Biological and Social Systems"—**Chairman**, William H. Taliaferro. **Speakers:** Libbie H. Hyman, American Museum of Natural History; James W. Buchanan, Northwestern University; Herbert S. Jennings, University of California at Los Angeles; and Ralph W. Gerard, William Burrows, Thomas Park, and Warder C. Allee, University of Chicago. **Special lecture:** Donald D. Van Slyke, Rockefeller Institute for Medical Research.

"Visual Mechanisms"—**Speakers:** Selig Hecht, Columbia University; Ernst Gellhorn, University of Illinois; Samuel H. Bartley, Washington University; Karl S. Lashley, Harvard University; and Arlington C. Krause, Heinrich Kluver, Theodore J. Case, and Stephen Polyak, University of Chicago.

"Levels of Integration in Biological and Social Systems"—**Chairman**, Robert Redfield. **Speakers:**

(Continued on page 52)

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris I. Gorokhoff and Judy Woodring.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879, and re-entered, July 23, 1938.

Introducing

Dr. Cheng-Kwei Tseng, Assistant Professor of Botany and Acting Curator of Herbarium, Lingnan University, China; University Fellow in Botany, University of Michigan.

A native of Amoy, China, Dr. Tseng has spent most of the last ten years collecting marine algae along the entire coast of China. He has worked on all phases of Chinese algology, including taxonomy, morphology, ecology and economic uses, and has published a number of papers in Oriental botanical and scientific journals on these subjects.

Upon completing his post graduate studies at Lingnan University in 1934, he was appointed lecturer in botany and curator of the herbarium at the University of Amoy. A year later he was made assistant professor of botany and curator of the herbarium at the National University of Shantung, Tsingtao, positions which he held until 1938, when he was appointed to Lingnan University. During part of this time he was collaborate algologist at the Marine Biological Station in Amoy. In 1937 he was connected with the newly established Marine Biological Station at Tsingtao, but the station was destroyed by the Japanese soon afterwards.

Last September he arrived in the United States to work at the University of Michigan under Dr. Wm. Randolph Taylor. He is particularly interested in correlating species of Chinese algae with those of the United States coasts. He considers it quite likely that a number of Oriental species of algae, which have been given separate specific names, are actually identical with American species, and, conversely, that species hitherto considered to be the same may actually be different. The only way to resolve these questions is to study specimens from both the Pacific and the Atlantic regions, preferably in the living condition. He has studied Chinese forms extensively, and is now working on certain American species for the purpose of comparison. In connection with this work, he brought part of his algae collection, amounting to about 3,000 specimens, with him to the United States.

He has also been working on monographs of certain Chinese algae, and has already published since his arrival in this country treatises on *Liagora, Wrangelia, Griffithsia, Codium* and *Chaetangiaceae.*

At Woods Hole this summer he is also assisting in the Botany course, together with Mr. W. J. Gilbert, taking the place of Dr. Rufus H. Thompson. Dr. Tseng will spend the academic year 1941-42 at the University of Michigan and will then return to China.

ADDITIONAL INVESTIGATORS

Aquila, (Sister) M. grad. biol. Villanova. Rock 3.
Bodian, D. asst. prof. anat. Western Reserve Med. lib.
Brown, D. E. S. prof. phys. New York. Br 310.
Calkins, G. N. prof. proto. Columbia. Br 331.
Chambers, E. New York Med. Br 343.
Chambers, R. prof. biol. New York. Br 328.
Duncan, G. W. fel. surg. Hopkins. Br 328.
Genevieve, (Sister) Mary grad. biol. Villanova. Rock 3.
Gilbert, P. W. instr. zool. Cornell. OM.
Lancefield, D. E. assoc. prof. biol. Queens (New York). Br 126.
Pick, J. instr. anat. New York Med. Br 343.
Rahn, H. instr. zool. Wyoming. lib.
Stebbins, R. B. grad. asst. biol. New York. Br 328.
Stiegelman, S. asst. zool. Columbia. Br 313.

SYMPOSIA AT THE UNIVERSITY OF CHICAGO

(Continued from page 51)

Clarence R. Carpenter, Pennsylvania State College and the School of Tropical Medicine (Puerto Rico); Alfred L. Kroeber, University of California; and Alfred E. Emerson and Robert E. Park, University of Chicago.

"Sex Hormones" — **Chairman,** Frank R. Lillie. **Speakers:** Edward A. Doisy, St. Louis University; John S. L. Browne, McGill University; and Carl R. Moore, Allan T. Kenyon, and Fred C. Koch, University of Chicago.

"Immunological Mechanisms"—**Chairman,** George F. Dick. **Speakers:** Linus Pauling, California Institute of Technology; Thomas M. Rivers, Hospital of the Rockefeller Institute; and William Bloom, Paul R. Cannon, and William H. Taliaferro, University of Chicago. **Special lectures:** Charles H. Best, University of Toronto; and Ernst W. Goodpasture, Vanderbilt University.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
July 12	7:00	7:21
July 13	7:47	8:10
July 14	8:35	9:01
July 15	9:23	9:55
July 16	10:15	10:50
July 17	11:07	11:45
July 18	11:58	

In each case the current changes approximately six hours later and runs from the Sound to the Bay.

ITEMS OF INTEREST

DR. AND MRS. CHARLES PACKARD will be at home to members of the Laboratory on Sunday, July 13, and on the two following Sundays, from four-thirty to six o'clock.

DR. ROBERT GEORGE BALLENTINE was granted a National Research Council Fellowship for the year 1941-42 to work on the chemical organization of the cell surface at the Rockefeller Institute for Medical Research in New York. He is assisting in the physiology course at the Marine Biological Laboratory.

DR. MARGERY MILNE has been appointed assistant professor of biology in the Richmond Division of the College of William and Mary.

DR. DOROTHY M. WRINCH, the English mathematician and theoretical biochemist, has been engaged jointly by Smith, Mt. Holyoke, and Amherst Colleges to conduct at each institution next year a series of seminars open to the members of the three faculties and their advanced students. Working under a grant from the Rockefeller Foundation, she is at present a member of the faculty of physical science at Oxford and a lecturer in chemistry at the Johns Hopkins University.

DR. EUGENE F. DUBOIS has been appointed professor of physiology at Cornell University Medical College, and head of the department of physiology and biophysics, not biochemistry and physiology as previously reported.

DR. JOHN H. NORTHROP, of the Rockefeller Institute of Medical Research, received the honorary degree of Doctor of Science at Rutgers University on June 8.

Next week the physiology class will have the following guest speakers: Dr. M. E. Krahl will speak on Thursday, and Dr. O. C. Glaser will talk on "General Physiological Problems of Growth" next Saturday. Guest lecturers who have spoken previously are Dr. L. V. Heilbrunn, whose topic was "Protoplasmic Viscosity" and Dr. A. C. Redfield who lectured on "Respiratory Proteins." Dr. E. G. Conklin lectured before the embryology class today on the mapping of eggs.

DR. ALMA G. STOKEY, of Mount Holyoke College, will give a paper at the botany seminar on Thursday, July 18. She will talk on "The Gametophytes of the Filmy Ferns." Last week Dr. F. B. Smith of the University of Florida spoke on "Types and Distribution of Microorganisms in Some Florida Soils."

DR. JOHN S. RANKIN, instructor in the invertebrate zoology course at the Marine Biological Laboratory, is being married to Miss Julia Penfield Smith, (Invertebrate, 1940), today in Rockford, Illinois.

MISS MURIEL VOTER, an instructor at Wheaton College, married Carroll Williams, next year a Harvard Fellow, on June 26th in Middlebury, Vermont. Both the bride and groom took the class in Invertebrate Zoology at the Laboratory in 1939.

MR. DAVID J. BRADLEY, son of Dr. and Mrs. Harold C. Bradley, was married on April 26 to Miss Elizabeth B. McLane in Manchester, New Hampshire. The couple will make their home in Madison, Wisconsin.

DR. ROBERTS RUGH and his family will be in New York this summer. Dr. Rugh is teaching a course in embryology in the summer school of the Washington Square College of New York University.

MR. W. R. DILLON, chief of the division of administration of the Fish and Wildlife Service, is visiting the station with his wife and son for the month of July. They are staying at the Fisheries residence.

Firms which held exhibits at the Marine Biological Laboratory during the past week included: The Macmillan Company (Mr. Harvey C. McCaleb); The Spencer Lens Company (Messrs. Charles Riley and Frank Muñoz); and Ward's Natural Science Establishment.

Choral Club rehearsals are continuing on Tuesday and Thursday evenings. An appeal has been issued for more basses and tenors. On Thursday night these parts were filled by a group of soldiers from the 181st Regiment, who, on the spur of the moment, joined in the singing.

The program of the phonograph record concert next Monday at the M.B.L. Club will be as follows: Handel, "Concerto in D for orchestra, with organ"; K. P. E. Bach, "Concerto in D for orchestra"; J. S. Bach, "Brandenburgh Concerto No. 6"; intermission; Gluck, (opera): "Orpheus and Eurydice."

The M.B.L. Tennis Club has appointed Dr. E. R. Jones as treasurer until this summer's elections, to replace Dr. T. K. Ruebush, who was called into service in the U. S. Navy. Dr. D. E. Lancefield, club president, announces that the beach courts are now available for playing.

PHYSIOLOGY CLASS NOTES

With our last week of lectures and supervised lab work just about over, we are all busily engaged in the scheming of some brilliant and rare bit of research with which to occupy ourselves next week. The problem of finding a problem is difficult enough in itself, but already some of us have reached a decision. Fred Coe, being a great peanut enthusiast, is considering the problem of peanut butter metabolism in crustacea. Bernie Shepartz, who suggested this to Fred, will assist in the experiments by first determining whether the crustacea like peanut butter or not. Frank Hartman, emerging from the depths of the cellar where he has been micro-manipulating all week, thinks he will study tissue regeneration, his apparatus for which will consist of one good bed, plenty of time, and an alarm clock set for supper. Ed Burns has become quite interested in the work with dogfish ghosts and so he plans to study the art of ghost-breaking with the aid of the Topper series as reference.

Just a word about our favorite delicacy—micro-manipulation. At the beginning of the week we handled the needles and pipettes as if we had received our early training stacking cord wood. By the end of the week at least a few members emerged from beneath a stack of broken needles with a triumphant air, proudly holding samples of their work. We enjoyed the work very much even if we did have to get down to such fine points.

On Tuesday we changed sections and each one got involved in something new. The second group always gets a break because the apparatus is usually still set up and at least one big headache is thereby eliminated. There also seems to be an advantage because advice is available from those who have gone before and know what not to do. Thusly have the new micro-manipulators learned that dirty needles and clogged pipettes cannot be cleaned with a handkerchief, while the new Van Slykers are still wondering about the best method for gathering up mercury from the floor. The new cytochemists received a list of eighteen "don'ts" made out by their passing fellows and Dr. Ballentine. It seems both the student and Dr. Ballentine had a little trouble, so the first "don't" reads:—"Don't break glassware, especially burettes (instructor please copy)."

Tuesday turned out to be a lovely (?) day for a picnic, so we found ourselves, as usual, listening to a lecture at 9 A. M. Dr. Fisher, while gazing out the window, was heard to say, "I'd even rather work than go on a picnic today." So the lobsters were placed in a tank where they fought all day, the rest of the food was hidden, and five minutes were appropriated to silent prayer for sunshine on Wednesday.

Dr. Kempton delivered the lecture, his subject being observations made from direct investigations of glomerular and tubular renal functions. On Thursday he spoke on "The Concept of Renal Clearance," and on Friday, "The Problem of Tubular Secretion in the Kidney." Dr. A. C. Redfield was our guest lecturer on Saturday, his topic being "Respiratory Proteins."

Many mistakes which we might have made this week were avoided through the kind offices of an *ex-officio* physiology instructor who managed to find time, despite the exigencies of his embryology course, to instruct us in all phases of physiology. We thank him. —*J. E. H.*

BOTANY CLASS NOTES

Last Sunday, Dr. Runk spread a huge quantity of delectable foods before a group of the younger botanists on their visit to his home in Nantucket. That trip was the highlight of the week—probably of the course, too—and will doubtless be remembered by everyone for a long while to come. Being invited to spend the entire day on the island, the class left on the early boat, according to plans made by the ever-efficient Nancy Bull.

Upon docking, their first experience was an inside view of Maria Mitchell's house, where the telescope with which she discovered her comet is still in its old place. A pleasant surprise was the display of fresh Nantucket flowers on exhibit there. After this, the group left in cars for Dr. Runk's house, three brave souls—Connie Stanton, Jean Enzenbacher and Bob Thorne—taking a wild ride via the dunes and moors, where the driver tried to prove that travel is much more exciting when one doesn't bother with roads.

Swimming, talking, and then the boatride home with that gorgeous sunset in the West was truly the end of a perfect day for the tired algologists who finally reached Woods Hole in the evening.

A notable casualty next day was Connie Stanton's *faux-pas* in spilling a new bottle of *Scrip* ink over the desk, herself, and the general upper end of the Botany lab, while Dr. Taylor, unperturbed at the mishap, calmly continued his lecture. 'Tis said the lady demonstrated admirable nonchalance by placidly powdering her nose, but that's unadulterated gossip.

Not being lazy, as are certain unmentionable classes at the M.B.L., the botanists spent the whole damp Fourth in getting acquainted with marine algae at Nonamesset Island and Spindle Rock. In case any morose individual needs cheering, the guaranteed remedy is a position on the docks somewhere watching the chain of six skiffs slowly towed through the Gutter amid cheers and catcalls from the *Nantucket*. Unanimously proclaimed the best trip yet, this excursion listed only

two interesting accidents—Babs Bayard's fatal slip at the end after so carefully keeping semi-dry, and Sam Salvin's graceful misstep on *Mytilus,* which lost him his whole bucket of algae, only part of which he retrieved. Mild excitement was caused when Jackie Waldron and Connie Stanton, rowing one skiff, tried to shanghai "C.-K." Tseng and Bob Williams by heading for New Bedford instead of Eel Pond. They soon decided, though that the project wasn't worth the effort, and then headed for home like good children.

Last Wednesday, the class took another trip on the *Nereis,* that time to Nashawena, Pasque and Naushon Islands. Pasque, it seems, proved the most exciting for Bill Gilbert's group, who were broad-minded enough in their search for algae to stop to appreciate a pair of deer that approached them fairly closely before gracefully bounding off again. Some of the city critters had never seen wild deer—imagine!

Finally and foremost, the week's activities were climaxed last Monday evening by some more of Dr. Taylor's color film—gorgeous enough to make any true botanist realize he'd not have to be an esthete, even, to appreciate his algal materials.

—*J. W.*

EMBRYOLOGY CLASS NOTES

Academic Life:

The past week has been spent on Echinoderms with Dr. Schotté, who dwelt at first on the illusive orange ring of the eggs of *Paracentrotus.* The structure of *Arbacia* eggs was explained, and their polarity. It was shown by various experiments the lack of relationship between cleavage patterns and the final development of the egg. In the middle of the week we had a lecture by Dr. Chambers, renowned for his work in microdissection. The removal of nuclear material from eggs is as easy for him as an appendectomy is for a surgeon. This particular lecture was on activation of eggs and the surprisingly small part the sperm plays in the whole process of fertilization.

We went on with Dr. Schotté on artificial parthenogenesis. The class as a whole obtained quite good results, showing that the female can carry on. For further reference, Ray has suggested that we read "This New Magnificent World," by Huxley, in which the future of the Test Tube Embryos can be utilized as our first line of defence.

Dr. Schotté inspired us with his talks on the regeneration of amphibian limbs, which has been his special field during recent years. He is planning to test his theories on mammals as he feels that some aspects of his field can be applicable to higher vertebrates. As an example, the transplantation of periostial tissue will regenerate bone.

Social Life:

As mentioned in our last issue, our soft-ball prowess is improving, as was further shown when the crew defeated us by only two runs, quite a contrast to our first game with them. If a week's active training did this, just think what another week will do. Time will tell the tale. (Editorially speaking we hope it will be so—we disclaim responsibility.)

A peculiar, but very familiar odor greeted us one morning as we came from breakfast. Apparently there had been a skirmish in back of Physiology. The unmistakable results were obvious: They suffered!!

The Glorious Fourth was ushered in on the third and finished up on the fifth, at the picnic. The Embryology Lab was blitzkrieged from the roof of the Brick Building by some belligerent soul who objected to the industrious way we were spending our National Holiday (we had a radio —even an inspiration for dancing—remember, Trink and George).

The climax of our social life reached a peak Saturday with the Embryology *PICNIC* at Tarpaulin Cove! We took *'Winnie'* and *Sagitta,* or rather they took us the long way 'round amid songs and merriment. The duel of boats between Stirling and Fred (being towed) was a jumping off point of hilarity for the rest of the day. Clams, lobsters and Dr. Goodrich's scientific carving of watermelon formed the nucleus of our excellent and filling meal. Carrying out the theory that fingers were made before forks simplified the mechanics of eating. Trink and Tom chose sides for soft ball in which the sea played an important part as left field. Much fun was had by all. A sleepy, sunburned crowd finally piled into the boats. The Pirates of the *Sagitta* stormed *'Winnie's'* stronghold, carrying off what food remained. Trink was the star performer—with two cans of beer in one hand and four sandwiches in the other. At that he almost missed the boat. Our hearts and hands to you—Neil—for a grand picnic—efficiently organized.

—*P. and K.*

THE SOURCE OF PANCREATIC JUICE BICARBONATE

(Continued from page 45)

when the gland works hardest it produces the most CO_2 and this is reflected in a higher bicarbonate content of the juice. Second, because the pancreas is one of the few tissues that contain the enzyme carbonic anhydrase. This enzyme catalyzes the reaction $CO_2 + H_2O \rightleftharpoons H_2CO_3$. Now if the metabolic CO_2 of the gland is to be converted to bicarbonate for the juice, the first step in the process would be its hydration according to the above equation. Thus the occurrence of

carbonic anhydrase in the pancreas may be looked upon as an indication that this enzyme is needed there in order to speed up the hydration of metabolic CO_2 for the production of bicarbonate for the juice. If carbonic anhydrase plays such a role in the pancreas then any changes in its activity should be reflected in the composition or the rate of flow of the pancreatic juice. Mann and Keilin have recently shown that the drug sulfanilamide is a specific and potent inhibitor of carbonic anhydrase activity. We therefore undertook a study of the effect of sulfanilamide injections on the composition of the pancreatic juice of dogs. Somewhat to our surprise no effect on the rate of flow or the bicarbonate content of the juice was observed even when the concentration of sulfanilamide in the juice itself reached a value of 64 mg%. This concentration is 200 times that needed to completely inhibit the enzyme *in vitro*. These results therefore indicated that carbonic anhydrase had nothing to do with production of pancreatic juice and suggested that the metabolic CO_2 of the gland was not an important source of the juice bicarbonate.

If this was the case then the bicarbonate of the juice must be derived mainly from the blood stream. In order to prove this we have made use of radioactive carbon (C_{13}). This was produced by bombarding boron oxide in the cyclotron. Bicarbonate containing this radioactive carbon was then prepared and injected intravenously into dogs. The distribution of this radioactive bicarbonate between the pancreatic juice and blood plasma could then be followed by measuring the radioactivity of samples of these fluids collected at various intervals after the injection. Now if all the bicarbonate of the juice is derived from the plasma, then we may expect to find the radioactive bicarbonate concentrated in the juice to the same extent as the total bicarbonate. If no juice bicarbonate is derived from the serum, then there should be no radioactive material in the juice. If the juice bicarbonate is a mixture derived from both plasma bicarbonate and metabolic CO_2 then the radioactive bicarbonate content of the juice should be in between these two extremes, and be a measure of the proportion of bicarbonate derived from the plasma. The results of four experiments uniformly showed that the radioactive bicarbonate appeared promptly in the pancreatic juice in a concentration four to five times that found in the blood plasma. The total bicarbonate of the juice in these experiments was also four to five times that of the blood plasma. The results therefore indicate that the bicarbonate of the pancreatic juice is largely derived from the blood stream.

The pancreas is thus able to effect a four to five fold concentration of the plasma bicarbonate. This raises the question: where does this concentration of bicarbonate occur—on passage of bicarbonate from the plasma to the pancreatic cells or on passage from the cells to the juice? Analysis of pancreatic tissue for bicarbonate shows that the millimols of bicarbonate per Kg of H_2O within the cells is 60% of that in the blood plasma (see Table I). Concentration of bicarbonate does not therefore occur on its passage from the blood stream to the cells, but on passage from the cells into the pancreatic ducts.

Table I.

Electrolyte distribution between blood plasma, pancreatic tissue and pancreatic juice.

All values expressed in terms of mM/Kg H_2O.

	Blood Plasma	Pancreatic Tissue	Pancreatic Juice	
			Slow Secretion	Rapid Secretion
Na	155.		158.	158.
K	5.4		6.0	6.0
Cl	120.	71.	108.	30.
HCO_3	28.	18.	34.	120.
			pH = 7.65	pH = 8.40

An explanation for this concentration of bicarbonate based upon the behavior of the chloride ion may be offered. As shown in Table I, the concentration of chloride within the pancreatic cells is also about 60% of that in the blood plasma. The pancreatic cell, unlike most tissue cells, would thus appear to be freely permeable to the chloride ion of the blood stream. The passage of chloride from the cells into the ducts would appear, however, to be a slow process since only when juice is secreted slowly does its chloride content resemble that of the blood plasma. As the rate of secretion increases, the chloride ion appears to be unable to diffuse fast enough to match against its share of sodium and potassium ions. The bicarbonate ion which appears to be more readily diffusible, therefore, takes the place of the chloride ion in order to maintain electroneutrality. There thus results a pancreatic juice high in bicarbonate and consequently possessing an alkaline pH which is more suitable to the action of the proteolytic enzymes which it carries.

(This work was performed with the collaboration of Drs. H. Tucker, B. Vennesland, and A. K. Solomon. This article is based upon a seminar report presented at the Marine Biological Laboratory on July 8.)

THE COLLECTING NET

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MAGNETIC MEASUREMENTS ON COMPOUNDS OF BIOLOGICAL SIGNIFICANCE

DR. L. MICHAELIS
Member, Rockefeller Institute for Medical Research

Diamagnetism and Paramagnetism are analogous to induced electric dipoles and permanent electric dipoles. In magnetism, there is no phenomenon analogous to a free electric charge. A further difference between magnetism electricity is the fact that electric moments, whether induced or permanent, may vary widely with respect to their magnitude, whereas diamagnetic moments are always very small, always involving repulsion by an external magnet pole; and paramagnetic moments, if they exist at all, are always relatively large and cause attraction by a magnet pole. All matter is diamagnetic, but in certain chemical compounds, paramagnetism is superimposed on diamagnetism, and being much larger, makes the latter almost negligible. It may be added that the phenomenon of ferromagnetism exhibited by a few metals, such as iron or nickel, practically only in the free metallic state, is never (*Continued on page 72*)

M. B. L. Calendar

TUESDAY, July 22, 8:00 P. M.

Seminar: Dr. G. H. Parker: "The Melanophore System of Teleosts."

Dr. R. L. Watterson: "Some Aspects of Pigment Deposition in Feather Germs of Chick Embryos."

Dr. H. L. Hamilton: "The Influence of Hormones on the Differentiation of Melanophores in Birds."

Dr. Hermann Rahn: "The Distribution and Development of the Melanophore Hormone in the Pituitary of the Chick."

FRIDAY, July 25, 8:00 P. M.

Lecture: Dr. Dorothy Wrinch: "The Native Protein."

THE MAPPING OF EGGS

A LECTURE TO THE EMBRYOLOGY CLASS

DR. E. G. CONKLIN
Emeritus Professor of Biology, Princeton University

I feel like apologizing for appearing before an audience of people who are doing so much important work in this day and age, because I have to report on things which were done long ago. For nearly fifty years past I have been asked to lecture before this class in embryology and almost always I have spoken on essentially the same subject, though not, of course, in the same terms or under the same topic.

I was once introduced as "the friend of the egg," and I am proud to claim that title. I have always maintained that the egg is after all one of the most marvelous things in the world and there is never an end to thinking about some of the marvels and wonders of development.

I am therefore to give you something of a historical review of the backgrounds of modern embryology. The marvel of development never grows old or stale. In a single night an ascidian egg will be-

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JEAN LOUIS RUDOLPHE AGASSIZ

Who 68 years ago founded the Andersen School of Natural History on the Island of Penikese, which was the forerunner of the Marine Biological Laboratory. The bust was unveiled in the Hall of Fame in 1928 and is the work of Anna Vaughan Hyatt.

come a swimming tadpole. At six o'clock in the evening it may be fertilized; at six o'clock in the morning it will be out of its egg membranes. In the case of *Molgula*, it will be swimming actively only twelve hours after fertilization.

In a few days an *Arbacia* egg is a swimming pluteus. In a month *Crepidula* becomes a fully formed, swimming veliger. In nine months the human egg becomes a baby. While some of us labor for years to bring forth a brain-child in an article or book, nature brings forth a man-child in nine months. The brain-child is a fleeting thing, the man-child is a link in the endless chain of life.

Development as we now understand it means both differentiation and integration. Differentiation is the becoming different of various parts. From the times of the ancient Greeks down to the year 1759 it was generally believed that this development consisted chiefly of growth of a previously formed organism. Hippocrates said that blue-eyed parents produced blue-eyed germs. That was the belief that was held generally. Indeed, it was thought to be necessary philosophically, logically and theologically. It was supposed that development must be the unfolding of an infolded organism and consequently it was not nearly so mysterious and wonderful a process as we now know development to be.

In 1680 Antony van Leeuwenhoek, with simple lenses—a simple lens in a copper plate—studied the spermatozoa of man and different animals and described them quite accurately. Other people, taking his study as a basis, claimed that the spermatozoa of man contained the homunculus, the little man. They reported it with head and body and arms and legs.

I was reading only yesterday in our excellent library, some of Leeuwenhoek's letters in the earlier volumes of the *Philosophical Transactions of the Royal Society*. He wrote 125 letters which were published in some of the first twenty-four volumes, and in these letters he says that his observations have been made sensational by other people. He knows perfectly well that there is no such homunculus in the spermatozoon. Furthermore he says we know that in earlier stages of development the embryo is spherical while these sensationalists maintained that the arms were stretched out and the legs straight. Yet he did say that the man was pre-determined in the sperm and that in the uterus the sperm merely found a place to develop. With the prevalent masculine psychology of the time, the seed was supposed to come entirely from the male, while the female furnished a place, a location for its development.

Well, this notion of the pre-determination of the man in the sperm was carried still further by van Leeuwenhoek in a letter which is not published in the *Philosophical Transactions of the Royal Society*, but which I came across in a Dutch publication discussing the work of van Leeuwenhoek. According to this, in 1680 he wrote and published the statement that the sperm are of two kinds, one male-producing and the other female-producing. When I showed this statement to Professor Wilson in 1905 or 1906, following his great discovery, he said, "Verily there is nothing new under the sun." This discovery of male-producing and female-producing spermatozoa was not actually made but was anticipated by Leeuwenhoek in 1680. Again those that were making a sensation out of his discoveries said that Leeuwenhoek had discovered boys and girls in the uterus. That was nonsense, he said, but there are male-producing and female-producing sperm. He was getting at the idea of development by differentiation as contrasted with pre-formation.

However, it was not until the middle of the eighteenth century that this doctrine of preformation was carried to such absurd lengths that it simply broke down of its own weight. The Genevan naturalist, Bonnet, was largely responsible for this. You will find a very fine account of Bonnet's work by Professor Whitman, in the "Lectures from the Woods Hole Laboratory," for 1896, I believe. Professor Whitman reviews the work of Bonnet and points out the beautiful work that he did but the very erroneous conclusions to which he came. The conclusions were that there was no development. The embryo was infolded and it merely unfolded. Between the halves of a peanut, you can see the little leaves and stem and root of a plant, and you will see how easy it was to believe that in the earliest stages there was the little plant or the little animal in the seed. And this conclusion might have stood even longer than it did, for in addition to Bonnet, it had the support of many others including the physiologist Haller, who said "There is no becoming", that is, the plant or animal has been there from the start. They reasoned that if there was a little homunculus in the sperm or in the egg, of course the little homunculus had sex organs and germ cells, and inside those there was the next generation, and

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inside those the next, and so the doctrine of *emboîtment* or encasement arose, and became so absurd that it had to be abandoned.

Then a young medical student, Gaspar Frederick Wolff, published his doctoral thesis in 1789, entitled "Theoria Generationis". He made an actual study of the hen's egg and of certain plant seeds and found that in the earlier stages there was no little animal or plant in the egg, but he found instead what he called globules. These globules we now call cells. He found membranes of some gelatinous material but no trace of an organism, no trace of a living thing. He considered that the development was a process of differentiation caused by forces in nature to which he gave the name *nisus formativus* or *vis directrix*. These forces from the outside, acting upon these globules or membranes brought about development, and therefore since they were from the outside the whole process was called epigenesis. The word epigenesis meant genesis upon the germ, from outside stimuli.

For a hundred years or more after this discovery of Wolff, the doctrine of epigenesis was universally accepted and was held to be absolutely true. In the lectures of Professor Whitman you will find his answer to the assertions of Professor Bourne of Oxford, who maintained absolute epigenesis, namely that there is no organization in the egg, and development comes from the outside. Whitman showed the absurdity of this. He asked how is organization going to be "cooked into the egg" which lacks it? What *nisus formativus,* what *vis directrix* is he going to demand to bring into the unorganized egg organization and capacity for development?

I have made a translation from a book which is in the library and which I wish you would see. It was by one of the eminent zoologists of a previous day, Alexander Goette. In 1874 he wrote a very large treatise, together with a volume of plates, entitled "Entwicklungsgeschichte der Unke," the "Development of the Toad," from which I will quote parts of pages 35 and 77. This is what Goette wrote in 1874:

> The egg of Bombinator igneus ready for fertilization is neither in whole nor in part, neither in origin nor in its developed appearance a cell, a living organism, but is merely an essentially homogeneous mass enclosed in an externally formed membrane, which first begins, through fertilization, to produce in itself living forms. But before this end is reached, the entire yolk mass and individual yolk pieces are lifeless transition stages from unorganized material to an actual organism.

Just think of that, as late as 1874 the egg was not a living thing!

In 1898, when Jacques Loeb announced his discovery here at this Laboratory of artificial parthenogenesis, the newspapers immediately took it up. "Jacques Loeb had created life! By putting an egg in a solution of magnesium chloride, he had created life." But of course the egg was just as certainly alive as is the animal into which it develops, though not as completely alive, for there are degrees of living.

In 1883 another distinguished physiologist, Pflüger, maintained that the egg of the frog is isotropic, that is in every direction from the center there are exactly the same substances and its cleavage cells are indifferent; they are all exactly alike with "no more relation of the cells to the adult body than the snowflakes have to the resulting avalanche." The snowflakes are not predetermined to form an avalanche and the cells of the egg are not differentiated for the building of the body of the animal.

In 1893 Hans Driesch, whose death last April has saddened all of his good friends in this country announced a similar conclusion. We knew him and admired him although in many respects his work was wrong, I had my difficulties with him in the matter of the ascidian egg. When I found that parts of the ascidian egg gave rise to only a partial larva, while Driesch maintained that such larvae were entire, I said that the difficulty was that Driesch did not recognize the difference between a half larva and a quarter one; if only these larvae had legs, Driesch would have seen that they were not complete. Driesch replied, "Conklin does not think well of experimentalists, but at least this much we know—the difference between a quarter and a half."

His doctrine was like that of Pflüger, that the egg was isotropic and all the blastomeres were equivalent, having no specific relation to developing organs. He said that every blastomere of the sea urchin egg is equipotential, i.e., it is capable of giving rise to the entire organism. *"Jedes kann Jedes."* Every cell can give rise to the whole organism, and again he said that blastomeres were "like balls in a pile", so that you can shift them around any way you please, and still they would develop normally. In order to account for normal development, he had to invent something like a *vis directrix*. Something of that sort was necessary to make equipotential cells differentiate into a normal organism; something had to intervene, presumably from the outside, and direct the development, and that something Driesch called, as you know, *entelechy*. The *entelechy* of Driesch has many resemblances to the *vis directrix* of Wolff, or the "perfecting principle" of Aristotle. While I have argued against this in certain writings in the past, maintaining that names of mysterious forces that you can't experiment with are no solution of a problem—Driesch said you can't experiment with *entelechy*—as I get older I begin to see the necessity of something in living

things that has not yet been explained by rigid mechanism. I don't doubt that sometime it will be explained, that sometime we will find that matter and energy are so constituted that they may account for all vital phenomena. I am greatly impressed with the fact that living things experience satisfactions and dissatisfactions. We know that we have satisfactions and dissatisfactions, and there are evidences that higher and lower animals also experience these. Even Paramecia, swimming in a trough of water which is hot at one end and cold at the other avoid extremes of heat and cold. Why? We would say because the water is getting uncomfortable. There is something subjective, internal in Paramecia that causes them to draw back from things that are uncomfortable. That may be called a tropism, or a negative reaction to extremes in temperature. Why do they have this negative reaction? There is something in the living substance that feels, something that corresponds to the subjective phenomena that we have. Don't you see, I am coming around a little to sympathize with Driesch and his *entelechy*. The future of biology will have to deal with these phenomena which we call subjective feelings—satisfactions. Maybe their causes can be found in chemical and physical phenomena, such as "affinity," saturation, equilibrium, etc. Well, you see, I am getting 'way off into speculative regions.

(*Concluded in Next Issue*)

PATHOLOGY AND IMMUNITY TO INFECTION WITH HETEROPHYID TREMATODES

DR. HORACE W. STUNKARD
Professor of Biology, New York University

The term heterophyid refers to a large family of digenetic trematodes which infect fish-eating birds and mammals. *Heterophyes heterophyes,* the type species, was discovered by Bilharz (1851) in autopsies in Cairo. These trematodes are world wide in distribution and manifest notable lack of specificity in their final hosts. Every species so far tested will develop in the common laboratory animals; many of them have been recorded from human hosts and probably all are possible parasites of man. Members of the family have lophocercous, oculate, monostome cercariae which encyst in fishes and so eventually reach their final hosts. Looss (1899) first pointed out that the infective larvae must be acquired by eating uncooked fish.

Yokogawa (1913) found a high incidence of infection with *Metagonimus yokogawai* in Japanese, and, following the dictum of Looss, discovered the metacercariae in food fishes of Japan. He traced the development of this species in experimental animals and by human autopsy. Yokogawa reported that young worms, after liberation from their cysts, penetrate the wall of the intestine where they develop to sexual maturity, after which they return to the lumen of the intestine and eggs of the parasite are voided with the feces. Essentially similar findings in experiments with related species were reported by Ciurea (1924) in Roumania and by Faust and Nishigori (1926) in the Far East. Ciurea emphasized the possibility of secondary infection of lesions produced in the intestinal wall by migration of the developing worms.

Africa and his collaborators, in the Philippines, found intramucosal invasion by various heterophyid species, but, according to these authors, the worms which enter the tissues do not return to the lumen of the intestine. Instead, they remain in the intestinal wall where they destroy the glands and erode the tunica propria but elicit only a mild or no inflammatory reaction. As a consequence, they are not walled off by fibrosis and their eggs pass into the general circulation. The eggs localize at various places, especially in the walls and valves of the heart. Autopsy of many cases of acute cardiac failure revealed extensive involvement of the heart, and these lesions were regarded as the immediate cause of death. Experimental infection of various animals yielded divergent results and led Africa and his associates to the opinion that when introduced into natural hosts, the young worms remain in the intestine and produce a transitory and relatively harmless infection, whereas when they are liberated in the intestine of unusual hosts, they find the conditions unsuitable and bore into the intestinal wall.

Cryptocotyle lingua is a common heterophyid species in the Woods Hole area, where it normally infects gulls, terns and various mammals. Its life cycle was reported by Stunkard (1930) who attempted to infect different experimental animals. The larval stages are produced in *Littorina littorea,* and *L. rudis,* while the cercariae encyst in the cunner and other fishes.

Stunkard and Willey (1929) studied the development of *C. lingua* in cats and rats. In these hosts, the worms developed to sexual maturity between the intestinal villi and no intramucosal invasion was observed. Since there is evidence to indicate that cats and rats are not favorable hosts, the studies were continued using terns and dogs. Results of these experiments, done jointly with Dr. C. H. Willey, were illustrated by lantern

slides. Young terns, removed from their nests soon after hatching, were kept in the laboratory and maintained on cunners heavily infected with cysts of *C. lingua*. They developed a very severe infection from the 6th to the 14th day, when the number of eggs in the feces began to diminish. After the 20th day the feces contained very few eggs and large numbers of young worms recently liberated from their cysts. The results of the experiment supplement and clarify observations on infection in nature. Young gulls and terns, yet unable to fly, are always heavily infected while adult birds, although subject to continual reinfection or if kept in confinement and fed thousands of cysts each day, seldom harbor more than 15 to 20 mature worms. It is apparent that after an initial heavy infection, gulls and terns develop a strong resistance to superinfection and the presence of a few worms serves to maintain a substantial immunity. In terns, the young worms, when they emerge from their cysts, penetrate between the villi and disappear. Casual examination of the surface shows few parasites but sections of the intestine show that the worms, although they invade deeply between the villi and cause much desquamation, rarely if ever enter the tissues. After the first heavy infection, when resistance develops and almost all of the parasites are shed, the intestinal epithelium is regenerated and the bird does not suffer any apparent ill effect.

The experiments with dogs yielded very different results. A dog, fed enormous numbers of cysts, began to pass eggs of the parasite on the 5th day. It was killed at this time and the intestine examined. Large numbers of immature and mature worms were present on the surface of the mucosa and in the crypts between the villous folds. The villi showed acute inflammatory changes, desquamation, hyperemia and excessive mucous secretion. There was no invasion of the intestinal glands or tunica propria. Another dog, similarly fed for 14 days, was in a moribund condition. Killed at that time, autopsy revealed the presence of thousands of sexually mature worms. Sections of the intestine showed a copious exudate, which in places almost filled the lumen. The exudate contained strands of tissue, pieces of sloughed epithelium, and numbers of worms. Many worms were present also between the villi, deep in the crypts of the mucosa. In places, they had produced pressure atrophy, marked hyperemia of adjacent villi, extensive desquamation and destruction of the villi. There was some leucocytic infiltration, accumulation of eosinophils and plasma cells, with regenerative hyperplasia of the intact epithelium. The erosion of the mucosa resulted in an acute catarrhal enteritis with proliferation of fixed tissue elements in the adjacent areas. No parasites were found in the tunica propria or in normal intestinal glands. Dogs were given a moderate infection and allowed to recover. Eggs began to appear in the feces on the 5th day, were numerous for about 4 weeks, after which the number began to decline. At the end of 3 months very few eggs could be found and the feces were negative at the end of 6 months. After resistance had been established in dogs, the feeding of large numbers of metacercariae produced no visible ill effects and very few eggs appeared in the feces.

These experiments show that birds and dogs, if the latter survive an initial infection, effect a "self-cure" (as that term was defined by Stoll, 1929) and thereafter are resistant to any substantial reinfection.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 15.)

FACTORS IN THE LUNAR CYCLE WHICH MAY CONTROL REPRODUCTION IN THE ATLANTIC PALOLO

Dr. Leonard B. Clark

Assistant Professor of Biology, Union College

Of all the physiological influences attributed to the lunar cycle, the coincidence of reproduction of certain marine Polychaetes with specific phases of the moon has been best determined. Of such animals, the palolo worms are perhaps outstanding because of their size, their striking reproductive behavior and the apparent specific relation between the moon's phases and time of reproduction.

The only palolo in the Western Hemisphere is the Atlantic palolo, *Leodice fucata*, found in Bermuda, the Gulf of Mexico and the West Indies. It is a burrowing form with the gonads limited to the segments of the posterior half of the worm except the last score or so. At reproduction the sexual segments break from the rest of the body and swim to the surface where they discharge their sexual products. There is generally typical mass activity so that many investigators concluded that the reproductive period is concentrated into a few days during which the sexual ends appear at the surface in immense numbers.

The work of Mayer, and Clark and Hess shows that at least three factors serve to determine the particular day on which the majority of worms reproduce. Water turbulence induced by wind action will inhibit swarming if the wind velocity

exceeds nine miles per hour unless the reef inhabited by the worms is protected.

Records of swarming, 1898 to 1939, show that reproduction occurs most commonly about the time of the third quarter moon, less commonly at the first quarter, seldom at the full moon and never at the new moon, during the period from June 28 to August 1. This indicates that in some way the third quarter moon is most effective, followed by the first quarter, full moon and new moon.

Worms will not reproduce at the third quarter moon less than 353 days from the date of reproduction of the previous year nor less than 369 days at the first quarter. The maturity of the worms determines the earliest date for reproduction. The difference in time of maturity at which swarming will occur at the third and first quarter moon phases is correlative evidence that the third quarter moon phase is more effective than the first quarter in inducing reproduction.

Hempelmann believed that the effect of the lunar cycle was indirect on *Nereis dumerlii*. He considered that tides changing with the moon caused changing nutritive conditions which brought *Nereis* into phase with the lunar cycle. Friedlaender suggested that changes in hydrostatic pressure due to tides controlled reproduction in the Pacific palolo, *Eunice viridis*. Neither of these hypotheses will hold for the Atlantic palolo. Mayer showed that animals in open floating cars reproduced at the normal time. Obviously there was no change in hydrostatic pressure. The change in nutritive conditions in transferring rocks containing worms from their natural habitat to live cars must have changed the nutritive conditions of the worms much more greatly than any change in tides. Also, rocks containing worms were scrubbed clean of the organic debris on which the worms feed, placed in floating cars and the worms swarmed normally.

Furthermore, shading the worms in live cars from moonlight for more than two days immediately before the time of swarming inhibits reproduction. These observations, first made by Mayer, were repeated recently in two successive years with similar results.

At the present state of our knowledge, it seems almost impossible to escape the conclusion that moonlight acts directly on the Atlantic palolo to determine the time of reproduction.

A number of experiments on artificially changing the light relations of the lunar cycle by illuminating or shading racks containing worms were undertaken. The results of all the experiments are consistent in that if the average duration of light is increased, reproduction occurs before the controls, and if the average duration of moonlight is decreased, the time of swarming occurs after the controls or not at all. It is concluded, therefore, that this is a factor involved in reproduction and the effectiveness of the various phases of the moon's cycle is correlated with the average duration of moonlight during the cycle.

However, if this were the only factor involved, the effectiveness of moonlight to induce swarming would increase to a maximum about three days after the full moon and then decrease. But the effectiveness of moonlight is bimodal, the modes centering about the first and last quarter moon, with the latter much more effective. Obviously there must be some other factor operating in moonlight. The only other factor varying in the desired manner is the daily difference in the rate of change of moonlight. This reaches a maximum at the new and full moons and a minimum at the first and third quarter. If it is postulated that the effectiveness of moonlight in determining the time of swarming bears some correlation to the average duration of moonlight during the lunar cycle and bears some correlation to the reciprocal of the difference in the daily rate of change of moonlight, the resultant varies in a manner similar to the incidence of swarming during the lunar cycle.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 15.)

METABOLISM AND FERTILIZATION IN THE STARFISH EGG

DR. HERBERT SHAPIRO
Department of Physiology, Vassar College

In his book "Artificial Parthenogenesis and Fertilization" (1913), Loeb summarized his views on the nature of fertilization by stating that "one essential effect of the entrance of the spermatozoon into the egg of the sea-urchin is the acceleration of processes of oxidation". Although this is a good generalization for the sea urchin egg, it is not valid as a generalization for all eggs. Whitaker demonstrated that in the eggs of the clam *Cumingia,* (J. Gen. Physiol., *15,* 183, 1931) and the worm *Chaetopterus,* (J. Gen. Physiol.,*16,* 475, 1933) there is not a rise, but a fall of respiratory activity on fertilization, and in making a survey of all available data, tried to abstract some common element. On plotting out the absolute rates of oxygen consumption of different eggs before and after fertilization, he observed (J. Gen. Physiol., *16,* 497, 1933) that while the rates were quite divergent before fertilization, they became much more nearly the same after fertilization, and

proposed that the alteration in rate at fertilization is such as to bring it down or up to a more or less common level, comparable with that of normally growing cells.

This thesis has in turn had to be modified owing to the discovery of Rubenstein and Gerard (J. Gen. Physiol., *17*, 677, 1934) that the value of the ratio, fertilized rate/unfertilized rate (F/U) is not a constant in the egg of the sea urchin, *Arbacia punctulata,* but depends upon the temperature, being highest at low temperatures. The significance of these findings is that the respiration-temperature dependence function must be different in fertilized and unfertilized eggs, and comes about through an alteration in the nature of the active enzyme systems responsible for overall oxygen uptake, which takes place on fertilization.

Earlier measurements (by Loeb and Wasteneys, (Arch. Entwicklungsmechn. Organ,. *35*, 555, 1912) and by Tang (Biol. Bull., *61*, 468, 1931) of the egg of the starfish *Asterias forbesii,* at a single temperature, had led to the conclusion that in this egg, there is no change in oxidative rate on fertilization. However, if one examines the original data, it is noted that relatively low percentages of cleavage were obtained and that the respiratory changes were in both directions. It seemed advisable therefore to extend these observations through measurements of eggs from a large number of individuals, and over a wide range of temperatures. Warburg respirometers were used, and batches of eggs which showed usually 90% or more development were obtained during May and June, which is the optimal season for starfish eggs. The respiration of unfertilized eggs may be constant for periods of over ten hours, whereas that of fertilized eggs is relatively constant at first, and then exhibits a gradually increasing rate as embryological development progresses. Measurements were made at about thirty different temperatures, between 11.5 and 27.8° C., and an average increase on fertilization of approximately 30 to 50% was obtained. The average value of F/U was not constant over the range investigated, but showed a slight decline with elevation of temperature. In view of these data we should include *Asterias* with other eggs showing metabolic change on fertilization, and not classify it as an exception.

On examination of the F/U-temperature curves for the eggs in which these data are available viz., *Sabellaria alveolata, Chaetopterus variopedatus, Arbacia punctulata* and *Asterias forbesii,* it appears that these cells show curves which indicate that the temperature dependence of the activity of the respiratory enzymes of unfertilized eggs is different from that of fertilized in all cases, and that hence there is an alteration in the nature of the oxidative enzyme system on fertilization. For *Arbacia punctulata,* this has been further demonstrated by various investigators by treating the eggs with agents known to affect these enzymes. In the other eggs, this conclusion is an inference from the temperature analysis, and further chemical studies are desirable.

By itself, therefore, the absolute direction and amount of change is probably not of primary importance, but it appears to be rather the nature of the enzyme system. The metabolic aspect of Loeb's generalization may be retained if we modify his original idea by stating that one of the essential features of activation is an alteration of the nature of the enzyme systems.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 8.)

BOOK REVIEWS

Fisheries Along Our Coast

NEW ENGLAND'S FISHING INDUSTRY. Edward A. Ackerman. Illustrated. pp. 294. $4.00. University of Chicago.

The great diversity of New England's fisheries can scarcely be appreciated until one reads Mr. Ackerman's account of this industry. In a straightforward easy to read style he has covered virtually every phase of the field, both at sea and on land. His thorough research covers not only the major aspects of the fishing industry but includes many details seldom presented and which add considerable interest and value to his work. To the layman many of these factors are little known, and even for those persons whose business is fishing, or the handling of fishery products, there is a rich store of information.

About one-half of the book deals directly with the various species of fish, mollusks and crustaceans, that are caught off the New England coast and landed in its numerous ports. Seasonal abundance, migrations, and value of catchall are adequately described as are fishing grounds, fishermen, gear, methods of capture, and the limitations and regulations that govern the fisheries. The description of the various types of gear used for catching fish, clams, quahogs, oysters, scallops, squid, crabs, lobsters, etc., is of especial interest. In the case of fish, the relative efficiency and seasonal productivity is given for weirs, pound nets, otter trawls, gill nets, trawl lines,

hand lines, and other devices. An account is given of the kinds of fish taken by such kind of gear.

The remaining chapters of the book give an account of the marketing and handling of fish and fishery by-products and of the economic aspects of the industry. They explain, for example, how the more perishable or the less desirable species can not compete in distant markets but how, nevertheless, fresh fishery products are penetrating farther afield because of improved transportation facilities and methods of handling. Also described is the role of the salt fish trade and the reasons for its great decline during the past few decades.

The author has included many illustrations, an index, and after each chapter a list of the more important references. There are likewise many tables giving the amounts and value of the New England catch in recent years, both domestic catches and importations, and in some cases these are segregated according to the methods of capture that were employed. Various points discussed are illustrated on a series of thirty-three charts. They include the distribution and magnitude of the catch of the more important species, fishing grounds, polluted areas along shore, location of weirs, and the location of canneries.

W. C. Schroeder

A Standard Text

WOODRUFF, L. L. "Foundations of Biology." pp. xvii + 773. New York, The Macmillan Company. 1941. $3.75.

New editions of classic texts are difficult to review impartially. The appearance of six editions in less than twenty years is clear evidence of intrinsic worth so that enthusiastic praise descends from the level of critical judgment to a politically expedient endorsement of public opinion. Adverse criticism is even more unfortunate for it can only be justified by laboriously selecting those slight blemishes which can be found in any work and then endeavoring to maintain the case that they are major faults. The soundest test is probably to inquire whether the volume still justifies its title.

Biology is a vast subject defined in the first paragraph of the book under review as "the science of matter in the living state". How far, then, does the volume continue adequately to describe the foundations of this science? This depends entirely on what the reader regards as the foundations of biology. Woodruff's view is very clear for in Chapter II "we now turn directly to the study of life itself in the only form it is known —the bodies of plants and animals". This leads to a discussion of the cell and its development into a multicellular organism, followed by three chapters on plants and four on animals. Then follow five rather conventional chapters on the comparative anatomy of organ systems leading to two regretably short, but thoroughly enjoyable, chapters on endocrine and nervous coördination which serve to link the previous descriptive biology to the subsequent more philosophical discussions. These start with the origin and continuity of life as an introduction to fertilization, development and genetics, pass logically to adaptation and the origin of species and the book ends with the humanistic and historical aspects of the science.

All this, of course, is superbly well done. Woodruff has evidently read everything which is worth reading and possesses the happy knack, shared by the honey bee, of re-presenting what he has digested in a form more palatable than it was before. In this he has been aided and abetted by an excellent artist (Miss Lisbeth Krause) and a publisher who has devoted unusual skill and intelligence to the design and production of the book. Criticism, however, still remains possible. If we accept "the bodies of plants and animals", i.e. comparative anatomy, as the true foundation of the study of life we are struck by the anomaly that less than one third of the volume is devoted to this study and that the remaining two thirds, while making pleasant reading, do not inflict a very rigid mental discipline on the student. No one knows better than Woodruff that the basis of all science is logic and that mathematics is the language of logic. Yet nowhere in the non-descriptive portions of the book does one find any warning to the freshman that, if he is successfully to continue his biological studies, he will be constrained to do so in a thermodynamical framework as rigid as that which surrounds even the freshman chemist or physicist. An introduction to the mathematics of growth, and an indication that it is possible for a species to have statistical validity, might well have been included among the "Foundations of Biology".

There is no doubt, however, that the sixth edition will retain the high rating among general biology texts which has already been gained by the previous five. *Peter Gray*

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris I. Gorokhoff and Judy Woodring.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879, and re-entered, July 23, 1938.

M.B.L. CLUB

The Annual Meeting of the M.B.L. Club will be held at the Clubhouse on July 21, 1941, at 7:00 P. M.

Order of business:
Minutes of last annual meeting.
Reports of officers.
Reports of committees.
Election of officers for the next year.
Consideration of proposed amendment to constitution.
General business.

All members are urged to attend this meeting.

Proposed Amendment to the Constitution: Article X shall be amended to read: "A building fund shall be maintained to be used for the repair of, or improvements to the Clubhouse. In this fund the Treasurer shall deposit each year 10% of the dues collected that year. The fund shall be administered by the Trustees and money withdrawn from it only by their action on request from the Executive Committee of the Club. Further, in accordance with an agreement with the Marine Biological Laboratory, the Treasurer shall pay to the Laboratory 25% of the dues collected each year."

At present Article X provides that 25% of annual dues be put into a building fund. In fact this payment has been used in repaying the laboratory for repairs and improvements made several years ago. A year ago the Club came into an agreement with the Laboratory whereby the Club is to regularly make a payment of 25% of its annual income from dues and the Laboratory will take care of the maintenance of the grounds, exterior, and structure of the building. This payment represents a payment of rent to the owners of the building. The Club will continue to be responsible for the interior of the Clubhouse. The second part of the amendment proposed will ratify this agreement.

The first part of the amendment as proposed will establish a new building fund that may be used for major repairs or improvements inside the Clubhouse, or for further enlargements.

Nominations for next year's officers will be presented at the meeting by the Nominating Committee, consisting of Drs. H. B. Goodrich, Samuel E. Hill, T. H. Bullock and J. Hutchens.

—*Sears Crowell*

ADDITIONAL INVESTIGATORS

Beck, L. V. instr. phys. Hahnemann. lib.
Boche, R. D. instr. zool. Pennsylvania. Br 221. D 112-A.
Bronfenbrenner, J. prof. bact. & immun. Washington Med. (St. Louis). Br 305.
Garner, H. res. asst. zool. Chicago. Br 332. Ka 22.
Goldinger, J. M. res. asst. med. Chicago. Br 125. K 14.
Gurewich, V. Cornell Med. lib.
Kopac, M. J. asst. prof. biol. New York. Br 311. A 106.
Kreezer, G. L. asst. prof. psych. Cornell. lib. D 312.
Mead, F. W. Ohio State. Br 111.
Metz, C. W. prof. zool. Pennsylvania. Br 304.
Morgan, Isabel M. asst. path. & bact. Rockefeller Inst. (New York). Br 320.
Reiner, J. M. biophysics (New York, N. Y.). lib.
Renshaw, B. asst. prof. zool. Oberlin. Br 218.
Sturtevant, A. H. prof. biol. California Tech. Br 126.
Warren, A. A. asst. path. Harvard Med. L 27.

A NOTE FROM DR. DURYEE

The following paragraphs are extracts from a letter received by Dr. Robert Chambers from Dr. William R. Duryee, assistant professor of biology at Washington Square College, New York University. Dr. Duryee was a reserve officer and is serving as lieutenant in the U. S. Army.

Your film was delivered to me in my truck just as my column was about to roll out on the road. We have been moving so often from one bivouac to the next that there has been no time to acknowledge your kindness in sending the film.

At present we are moving the 27th Division back from Tennessee to Alabama. What with 28 new 2½ ton 6 × 6 "ten-wheelers" and new 1½ ton and command cars along with the old, our regiment looks quite respectable out on the road—about three and a half miles long. As transportation officer I am charged with shuttling our Regiment and several battalions of Infantry. We go day and night for four days. It is good fun for me but pretty hard on the drivers who alternate 2 hours sleep and two driving.

Now that our first big maneuvers are over, with all the dust, "jiggers" (by the way what is the biology of these mites?) and lack of water, there will be quite a let-down. Most of the boys will get ten-day furloughs. Our Colonel has emphasized the need for interesting lectures, films, training of any sort for this in-between period. Your film is therefore exceptionally welcome. Can you send three more 16 mm? Capillaries, microdissection, mitosis—any and all are needed.

Can it be that Woods Hole and the M. B. L. still exist? I feel rather remote from cell physiology; but two days ago I found some lizard eggs in an old log and dissected them for some of my company. The lizards were well-developed with large yolk sacs attached and motility had set in. I wish I had these while teaching embryology.

CURRENTS IN THE HOLE

Date	A. M.	P. M.
July 19	12:39	12:50
July 20	1:30	1:43
July 21	2:20	2:31
July 22	3:05	3:15
July 23	3:49	4:00
July 24	4:30	4:43

ITEMS OF INTEREST

DR. AND MRS. CHARLES PACKARD will be at home to members of the Laboratory tomorrow and next Sunday from four-thirty to six o'clock.

DR. CURT STERN has been promoted from associate professor of zoology at the University of Rochester to professor of experimental zoology.

DR. CARL G. HARTMAN, of the department of embryology of the Carnegie Institution of Washington at Johns Hopkins University, has been appointed professor of zoology and head of the departments of zoology and physiology at the University of Illinois beginning September 1.

DR. JOHN B. BUCK, instructor in zoology at the University of Rochester, has been promoted to an assistant professorship.

DR. E. E. REINKE, head of the Department of Biology at Vanderbilt University, has been named Chairman of the Division of Natural Sciences of the University.

DR. H. H. PLOUGH presented a lecture at eight o'clock on Thursday evening to the class on embryology. He spoke on "Genes and Development."

DR. CASWELL GRAVE will be the guest lecturer before the embryology class on Tuesday, speaking on "Ascidian Metamorphosis."

Guest speakers before the physiology class next week will include Dr. Samuel E. Hill, who will speak Monday on "The Action Current of *Nitella,*" and Dr. E. S. Guzmán Barrón, who will lecture Tuesday on "Oxidation-Reduction Systems in Cellular Respiration."

DR. WM. RANDOLPH TAYLOR will give a talk at the botany seminar on Thursday evening entitled "The 1934 Allan Hancock Expedition."

DR. VICTOR JOLLES died in Madison, Wisconsin, on July 5. He was formerly on the staff of the Kaiser Wilhelm Institute in Berlin, and later for two years (1937-1938) at the University of Wisconsin. He is well known for his work on *Dauermodifikationen* in Protozoa and later for work on heat-induced mutations in *Drosophila.*

The 1941 Conference on Spectroscopy and its Applications will be held at the George Eastman Research Laboratories at the Massachusetts Institute of Technology from July 21 to July 23.

Among the exhibits in progress at Woods Hole this week are those of the Bausch and Lomb Optical Company at the Coast Guard Canteen, the Clay-Adams Company at the Old Lecture Hall, and the Macmillan Company in the Lobby of the Brick Building.

MISS ELEANOR BLEVINS is being married today to Dr. Donald J. Zinn, who has worked at the Laboratory in past years, at St. Barnabas Church in Falmouth.

MISS DOROTHY WELLINGTON, who is doing research work at the Laboratory, was married on June first to Dr. K. L. Osterud at her home in Melrose, Massachusetts. Dr. Osterud, who is from Ashton, Virginia, received his doctor's degree this past June from New York University, is working at the Marine Biological Laboratory this summer, and this coming academic year will teach at the University of Minnesota.

DR. DETLEV W. BRONK, director of the Johnson Foundation for Medical Physics at the University of Pennsylvania, and Dr. H. S. Gasser, Director of the Rockefeller Institute for Medical Research, spent last weekend at Woods Hole.

DR. PAUL A. WEISS, associate professor of zoology at the University of Chicago, visited Woods Hole on July 11 and 12. He is teaching at the University this summer.

DR. LEONOR MICHAELIS left Woods Hole with his family for northern New England yesterday.

MISS LOUISE E. BOYDEN, editorial assistant on the *Biological Bulletin,* returned to Woods Hole this week after a two-week trip to Maine where she visited Mrs. H. B. Neal at the Mt. Desert Biological Laboratory and afterwards stayed at Penobscott Bay.

At the Dartmouth Conference, the following men were elected to the executive committee of the Society for the Study and the Development of Growth: Dr. Paul A. Weiss, *chairman;* Dr. K. V. Thimann, *secretary;* Dr. J. W. Wilson, *treasurer;* Dr. B. H. Willier, Dr. O. L. Sponsler and Dr. E. W. Sinnott.

The program for the weekly phonograph record concert at the M.B.L. Club next Monday is as follows: Hindemith, "Trauermusik"; Shostakovich, "Symphony No. 1"; intermission; Wagner, "Parsifal—Prelude and Good Friday Music"; Wagner, selections from "Gotterdämmerung."

A "poverty dance" will be held at the M.B.L. Club tonight, at which dancers will wear rag costumes.

The United States War Department has placed an order for 52,000 laboratory mice with the Jackson Laboratory at Bar Harbor, Maine. These mice will be used for medical purposes in the national defence program.

ITEMS OF INTEREST

The ketch *Atlantis* of the Woods Hole Oceanographic Institution sailed on July 10 for a cruise to the continental shelf just south of Georges Banks to take borings of the ocean bottom under the direction of Dr. Henry T. Stetson, research associate in paleontology at the Harvard Museum of Comparative Anatomy. Dr. Theodore White, Fred Phleger and Walter Hamilton make up the remainder of the scientific party. The ship is expected to return about July 20. Early this week the power boat *Anton Dohrn* sailed for a routine trip under the supervision of Mr. F. Fuglister.

Dr. and Mrs. Thomas P. Hughes are spending the month of July in Woods Hole on their 30-foot motor cruiser anchored in the Eel Pond. Dr. Hughes is a member of the field staff of the International Health Division of the Rockefeller Foundation and for the past two and one-half years has been studying the epidemiology of yellow fever in the Uganda Protectorate, Africa. He and Mrs. Hughes will return to Uganda for further work if war conditions permit, and plan to sail from New York on August 20.

MAGNETIC MEASUREMENTS ON COMPOUNDS OF BIOLOGICAL SIGNIFICANCE

(Continued from page 61)

encountered in chemical compounds of biological interest.

All chemical compounds with all their valences saturated, and containing only atoms with completed electronic shells, are diamagnetic. Therefore the overwhelming majority of organic compounds is diamagnetic. The main interest lies in paramagnetic molecules. They arise under two conditions. The first case is represented by compounds containing atoms with uncompleted electronic shells. Of these, iron is most important for the biologist. The second case is represented by chemical compounds, especially organic ones, such as possess an odd number of electrons, in other words, compounds with a free, not occupied valence, usually designated as free radicals. All organic substances that can be reversibly oxidized and reduced, develop such a free radical as an intermediate step of oxidation-reduction. This very property produces the reversibility of oxidation-reduction. The existence of such free radicals in reversible dyestuffs such as methylene blue, pyocyanine, the yellow respiration ferment, has been previously demonstrated by a potentiometric method, surveyed last year by the author in one of the Friday-evening lectures in Woods Hole. The detection of paramagnetism during the process of reduction of such dyestuffs is another method to prove the existence of free radicals. This phenomenon is demonstrated by the lecturer in some lantern slides showing the appearance, and, later on, the disappearance, of paramagnetism during the process of reduction.

Among the iron-containing compounds it is essentially hemoglobin and its derivatives which may show paramagnetism. The magnitude of the magnetic dipole moment can be utilized to elucidate the chemical structure, especially the nature of the chemical bond between the iron atom and the surrounding complex-forming groups. Pauling and Coryell discovered that hemoglobin is strongly paramagnetic, whereas oxyhemoglobin is not paramagnetic at all but shows only the slight diamagnetism characteristic of all non-paramagnetic substances. The speaker extended this study to crystallized catalase from beef liver. The method had to be refined and a differential micromethod has been developed, which was in principle demonstrated in lantern slides. This method showed that catalase is strongly paramagnetic, yet not quite as strongly as hemoglobin, and corresponding more to that of alkaline methemoglobin such as found by Pauling and Coryell.

The cause of paramagnetism in free radicals is the spin of the odd electron, which is equivalent to a circular electric current. In ordinary valence-saturated molecules, the electrons are arranged in pairs, each consisting of two electrons with opposite spin, cancelling the magnetic effect of the spin. In molecules containing iron, the paramagnetism is due to the fact that there is an uncompleted electronic shell. In this case it is not necessarily true that the electrons are arranged in pairs with opposite spins. Some of the electrons may possess spins in equal direction. In this case, the paramagnetic effect of an electron is not cancelled but even increased, each of the electrons with parallel spins contributing its share. In other cases, the electrons may be paired. In oxyhemoglobin, all electrons are paired; hemoglobin contains five unpaired electrons with parallel spins. Catalase has four unpaired electrons. One should remember that the magnetic effect itself may not be of much significance, but the knowledge gained from the magnetic measurement in regard to the nature of the chemical bond will probably be of great value for the analysis of the chemical structure of such compounds.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 8.)

PHYSIOLOGY CLASS NOTES

This week Dr. Ballentine, the last member of the staff to deliver lectures, spoke on "Ultra-Micro Analyses for Biological Investigation," "Recent Advances in Histo- and Cyto-Chemistry," and "Enzymes as Analytical Tools." Dr. M. E. Krahl was the guest lecturer on Thursday, his subject being "Interactions of Biologically Significant Substances in Surface Films."

Dr. Ballentine is reported to be suffering from writer's cramp due to his failure to use free-arm movement while filling two blackboards with references. Tuesday's lecture was enlivened by a rare demonstration of what almost amounted to sky-writing. The janitors having considerately sponged away his carefully written blackboard lecture supplements, Dr. Ballentine with admirable aplomb mounted the front table and replaced them amid the cheers of the three members of the class who were awake by that time.

After a week of spirited competition in the high breakage derby, your correspondent and partner were successful in carrying off the top honors. Mr. and Mrs. J. Broken Van Slyke (the J. standing for Just) are now at home to visitors in Room 10.

Second prize was awarded to the *Limulus* blood group for their successful studies of disintegration processes in the Haldane-Henderson apparatus. Individual honors were given for special effort to Herb Weiner by the contest judge, Mr. Ino Hoodonit.

This week's issue finds the class excused from formal instruction and free to pursue the bypaths of individual research. At this time next week we expect to be able to report all sorts of discoveries.

This week's issue also finds Dr. Fisher learning to use the slide rule so that he can calculate in a hurry the results he got.

The Physiology High Command in a recent communiqué announces the loss of two runs in a crucial engagement in the Park Street sector, in which a valiant stand was taken against the crew division by Gunner Bob Harrison and Field Marshals Ed Burns, Dr. Kempton, Dick Henry, Jim (Ersatz) Green, Dr. Kenneth (Panzer) Fisher, *et al.* An impressive mass demonstration was staged by a crowd of non-combatants. In other words, we had a very excellent cheering section with banners and everything.

The hidden talent of one of our class members, Pat Perkins, has finally come to light in the contribution of the following *Ode to Arbacia*:—

The life of a Physie
Ever so busy
Is incomplete
Sans Arbacia neat.
We praise ya,
Arbacia.

—*Mr. and Mrs. J. B. V. S.*

BOTANY CLASS NOTES

The halfway mark is passed; five weeks are behind us, and only one is left. What has this time been to the botany students? Weeks spent investigating the *Clorophyceae*—the green algae, in which chlorophyll is the dominant pigment—and the *Phaeophyceae*—the browns, in which the chlorophyll is masked by the accessory pigments carotin, xanthrophyll, and fucoxanthin. Now the class is studying the third big group, the *Rhodophyceae*—red algae, most colorful because of the superabundance of phycoerythrin.

Studying the algae involves 8:30 lectures every morning by either Dr. Taylor or Dr. Runk. The rest of the day, to say nothing of the wee small hours of the night, is spent in lab where Mr. Gilbert and Mr. Tseng are kept busy running from table to table, assisting in the identification of species which seem so similar to the untrained students. Each collecting trip is bound to reap several of the innumerable species of *Enteromorpha* and *Polysiphonia,* and many a student has been disappointed to find that his cherished mount of *Enteromorpha minima* is nothing but *E. intestinalis* which he found on the first trip and has mounted every time since. Dr. Taylor, however, is still optimistically hoping that at least a quorum will cease trying to elevate the weed, *Ceramium rubrum* to a rare genus before the end of the course.

A week ago Wednesday, the collecting trip was to Penikese Island, and, as usual, the *Nereis* left Eel Pond at 8:30 a.m., with the lazy members of the class just making it. Luck with good weather held, so that the trip to the island and the walk around it were most enjoyable. Due to a high wind the day before, there was a rich wash of "seaweed" along the shore, with the result that many species not previously found were picked up in sorting out the drying algae. Only three pieces of the feathery *Plumaria plumosa* were found, however, which made the lucky finders prey to the ravenous sink-collecting scavengers that night in lab. Another rarity was the beautiful autumnal oak-leaved *Phycodrys.* All truly

spirited collectors had a great time getting thoroughly soaked in grabbing for algae in the quiet second between breakers on the windward side of Penikese. Once a leper colony and formerly the original M.B.L. station, the island is inhabited now by only gulls and terns, whose nests are so numerous that to cover the island without stepping on the protectively colored eggs was a real feat.

Dr. F. B. Smith, agronomist and professor at the University of Florida, had charge of the Botany seminar last Thursday night. His lecture was on the microorganisms found in the Florida soils. In addition to generally telling about the countless and varied inhabitants of earth, he brought out the fact that their presence largely determines the quality of the soil; hence, Florida ground, which has little life per square inch as compared with that further north, is quite poor for cultivation. After the seminar, his wife served delicious date cake and whipped cream—there went the figures! —with real Chinese tea, prepared by Mr. Tseng.

All-day picnics, it seems, with boats for transportation are apparently the vogue in this neck of the woods, but since the Botanists had already visited most of the island spots—via boat—on field trips, they made their class picnic last Tuesday a late afternoon and all-evening affair to the sand dunes near Sandwich. Upon arrival, everyone went either swimming or sliding down dunes in search of wood, then spent two and a half hours singing around the bonfire after a huge meal. Sufficient indication that the affair was a success was the remark made by Dr. Taylor, a confirmed picnic-hater, "It wasn't half as bad as I expected it to be!"

Getting back at twelve p.m., however, had no noticeable effect on anyone but Father Stenger who failed to put in appearance by 8:30 the next morning when the *Nereis* set out for a collecting trip to Gay Head. As is the customary technique, the three skiff-loads of algologists waded through the wash around the Head for several hours before returning to the boat for lunch. Collecting, nevertheless, was not too profitable—except to slow-pokes who still had neither *Laminaria* nor *Cystoclonium*—and though the shore waters were laden with algae, few new things were picked up. Later homeward bound, the *Nereis* stopped in two places to make dredgings, but, unfortunately for the vegetarians, except for a little *Phyllophora broadaei*, the total haul included only starfish, bryozoans, shells, pebbles, and hermit crabs. In spite of this, and since it's always fair weather when the botanists are together, no one minded much and the trip was set down as an even better one than last week's. —*J. W. and C. S.*

EMBRYOLOGY CLASS NOTES

Academic Life:

On Tuesday, July 8, Dr. Schotté completed his career at Woods Hole in a blaze of echinodermal glory when he discussed differentiation of chemical material within the egg as an example of the influence which the SO_4 ion has in the polarity of the egg. We are sorry that this course will no longer have the privilege of Dr. Schotté's inspiring lectures. This vivacious individual is planning to settle down to hard research (with no distractions) in the hills of Massachusetts.

Dr. Costello opened the discussion on Annelids with a detailed description of the future of each blastomere. We were all a little relieved when the *Nauplius* was finally produced with its three pairs of appendages and a simple eye spot instead of the complicated cell system which preceded.

On Thursday we were on the receiving end of a classical lecture by Dr. Hamburger which concerned primarily his own work in the field of experimental Neuroembryology.

Friday came cell lineage and our minds were spun with spiral cleavage. Without Ray's beautifully stained slides of *Crepidula* cell stages we never would have been unwound.

On Saturday, we had the pleasure of hearing Dr. Conklin, who gave an introduction on the argument of Preformative vs. Epigenetic Development and concluded with a brilliant discussion on "The Mapping of Eggs." This lecture was one of the outstanding features of the course and a memorable experience for us all to have had the opportunity of hearing the man who fathered the School of American Experimental Embryology. The "Friend of the Egg" amused us all with his account of the "cell lineagists" and the "egg shakers" at this Laboratory.

Monday, under the guidance of Dr. Hamburger, the Trochophore larva was studied with an air of semiaccuracy midst a cloud of subdued profanity. The little blighter twister and turned and managed to conceal all vital structures beneath yolk and array of pigment.

Social Life:

The high spot occurred Tuesday when one of the beautiful days, highly valued because of their rarity, called forth a group of truants who, though they departed through the back door of the laboratory were unable to escape the vigilance of Dr. Costello. With face wreathed in smiles the good doctor bade them farewell with a "bon voyage" and a mild suggestion that the laboratory be continued at sunset. —*P. and K.*

WOODS HOLE
GOLF CLUB
WOODS HOLE
GOLF CLUB
TOWN LANDING
PUBLIC PARK AND BALL GROUND
MILL POND
EEL POND
TOWN LANDING
BATHING BEACH
THE BREAKWATER
TOWN LANDING
COAST GUARD
U.S. FISH COMMISSION
MARINE BIOLOGICAL LABORATORY
WOODS HOLE OCEANOGRAPHIC INSTITUTION
HARBOR
TOWN LANDING
WESTERN UNION
RAILROAD STATION
STEAMBOAT
NEW BEDFORD
MARTHA'S VINEYARD
NANTUCKET
POST OFFICE
LIBRARY
TOWN LANDING
MAGNETIC AND TRUE NORTH
PLEASANT ST
WATER ST
MAIN ST
NORTH ST
WOODS HOLE
FALMOUTH
VINEYARD HAVEN

THE COLLECTING NET

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THE ORGANIZATION OF THE MELANOPHORE SYSTEM IN BONY FISHES

DR. GEORGE HOWARD PARKER
Professor of Zoology, Emeritus
Harvard University

The common eastern catfish, *Ameiurus nebulosus,* can vary in color from pale yellowish green to coal black. This color change, which depends upon the melanophores in the skin of this fish, is excited through at least three receptors, the dorsal retina, the ventral retina, and the skin. The color changes in *Ameiurus* take place slowly and require for their completion more than a day. Consequently this fish is especially favorable for color work.

The pale phase of the catfish is dependent upon the dorsal retina and is best seen when the creature is over a white background illuminated from above. Under such circumstances that light which passes directly from its source to the fish's eye enters that organ somewhat obliquely and, after passing across its interior, impinges upon the ventral retina. Of the light that falls on the white background below the fish and is reflected (*Continued on page* 89)

M. B. L. Calendar

TUESDAY, July 29, 8:00 P. M.

Seminar: Dr. T. C. Evans and Mr. J. C. Slaughter: "Radiosensitivity of Arbacia Sperm under Different Conditions."

Dr. J. P. O'Brien: "Effect of X-Radiation on Regeneration in Nais paraguayensis."

Dr. A. M. Chase: "Effect of Azide on Cypridina Luciferin."

FRIDAY, August 1, 8:00 P. M.

Lecture: Dr. Ernst Mayr, "Speciation in Birds."

STORAGE AND DISTRIBUTION OF MANGANESE IN *OSTREA VIRGINICA*

DR. PAUL S. GALTSOFF
U. S. Fishery Biological Laboratory
Woods Hole, Mass.

The tissues of marine Lamellibranchs store relatively large quantities of heavy metals. Fe, Cu, Mn, Zn and sometimes Pb were found in their bodies and it has been established that there is considerable variation in the first two of these elements in the oysters taken from various localities along the Atlantic and Gulf coasts. Copper was found to be particularly abundant in the oysters from the northern states whereas those from the South Atlantic and Florida waters are richer in iron.

The existence of definite seasonal changes in the glycogen and water contents of oysters and clams suggested that there may be similar seasonal fluctuations in the contents of heavy metals of these mollusks. To avoid variations which might have been attributed to racial differences, age, or location, oysters of known age and origin were planted on the experimental bottom in Long Island Sound near Milford, Connecticut. The

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STAFF AND STUDENTS OF THE PHYSIOLOGY COURSE AT THE MARINE BIOLOGICAL LABORATORY, 1941

Front row: Adele P. Schlosser, Patricia J. Perkins, Ingrith Deyrup, L. Constance Tuttle, Jane Henry.

Second row: Harold Gordon, Bernard Schepartz, Glenn H. Algire, James W. Green, William S. Keezer, Frank Hartman.

Third row: Herbert M. Weiner, H. Duncan Rollason, Jr., Joseph R. Stern, George Zimmerman, Philip B. Lorenz, Dr. Rudolph T. Kempton, Dr. Robert Ballentine, Dr. F. J. M. Sichel.

Back row: Jack Jacobs, Eugene Rosenblum, John R. Gregg, Dr. Kenneth C. Fisher, J. Edward Burns, Jr., Frederick W. Coe, Robert W. Harrison, Richard Henry, Richard Egan, Dr. Arthur K. Parpart, Dr. C. Ladd Prosser, Joseph Lein.

3000 bushels of oysters planted on this area represented a homogenous population from which for a period of about 3 years random samples were taken at bi-weekly intervals. Each sample consisted of ten oysters which were opened, weighed and placed in an oven for drying within three hours after they were taken out of water.

Employing the procedure recommended by Richards (*The Analyst,* 1930, 55:554) the samples were analyzed for Mn and other metals. The results revealed great regularity in seasonal variations of the manganese content of these samples. The lowest concentration of Mn (between 7 and 11 mg. p.k.d.w.) occurred during cold months (November-April) whereas the highest concentrations (between 35 and 55 mg. p.k.d.w.) coincided with warm seasons (May-August). A study of the sexual cycle of the oyster shows that during the latter period gonads undergo rapid development and the mollusks reach sexual maturity. Following the discharge of sex cells the Mn curve rapidly drops to its minimum. The regularity of the Mn-curve suggests that accumulation of this metal is somehow associated with the sexual phase of the oyster. This view is corroborated by the analysis of separated tissues of the oyster. For this determination samples each comprising 10 or 20 female or male oysters were prepared by excising the mantle, gills, gonad, muscle and visceral mass. The latter part always contained a small amount of gonad tissues which could not be completely removed. It has been found that ovaries had highest Mn content, varying from 51 to 59.6 mg. p.k.d.w. On the other hand the testes were very poor in Mn, having only from 4.6 to 7.3 mg. p.k.d.w. In the gills the Mn varied from 17 to 18 mg. p.k. in winter and from 35 to 38.6 in summer. In the visceral mass the Mn varied from 8.9 to 18.4 mg. p.k. and in the adductor muscle its content in July was 4.3 to 5.2 mg. p.k. and 4.1 to 9.3 in January. The mantle had a Mn-content of 8.7 mg. in January and from 14.2 to 17.0 mg. p.k.d.w. in September.

Since the ovary is obviously the principal organ in which the Mn is stored, it is reasonable to conclude that high content of this metal in mixed samples is primarily due to the presence of ripe females. The Mn cycle in *Ostrea virginica* is obviously associated with the development of the female phase. The exact role of this metal in the metabolism of this mollusk and its possible effect on sex change is not known. It is reasonable to expect, however, that being a strong catalyst, its presence in the ripe female has physiological significance which we hope will be elucidated by our further experiments.

Large quantities of Mn were found by Bradley in fresh water mussels (*J. B. Ch.* 1907 and 1910, V. 3 and 8) and Dubuisson et Van Heuverswyn (*Arch. d. Biol.,* 1930, 41) have shown that in *Anodonta cygnea* it is principally stored in the internal demibranchs. Its physiological role in mussels is not known.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 15.)

THE MAPPING OF EGGS

DR. E. G. CONKLIN
Emeritus Professor of Biology, Princeton University

(Continued from last issue)

I have spoken about embryologists who believed in extreme epigenesis, under the influence of *vis directrix* or *entelechy.* But coincident with their work was that of others who found that the egg and its blastomeres were not isotropic and equipotential. In 1874, the same year in which Alexander Goette published his work on the toad's egg, Wilhelm His studied the hen's egg and concluded that the surface of the blastoderm could be mapped, certain portions being destined to give rise to the head and others to other parts of the embryo, that is, that the egg could be mapped into *Organbildende Keimbezirke,* organ-building germ regions.

In 1877 Professor Whitman, afterwards the first director of this Laboratory, published his doctoral thesis on the embryology of *Clepsine.* While it was a little crude as compared with some of our modern work, it was very carefully done. He found that individual blastomeres gave rise to particular parts of the embryo, and he said that while the embryo is not predelineated in the egg, all its parts are predetermined.

In 1879 Carl Rabl studied the embryology of *Planorbis* and found that individual cells gave rise to particular parts of the embryo. Edward van Beneden in 1884 did the same thing for the ascidian egg, pointing out that the very first cleav-

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age is in the plane of bilateral symmetry, one blastomere giving rise to the right half of the body and the other to the left. That was an important discovery, to find bilateral symmetry established at this early stage.

In 1887, a young French student, Louis Chabry, studied the ascidian egg, following the methods of Driesch. He found that one of the first two blastomeres gave rise to half a larva. Driesch maintained that it was a whole larva, but his pictures of ascidian larvae were very crude and inconclusive.

In 1888 Wilhelm Roux stated that the frog's egg from the four-celled stage on is a "mosaic work", each of these portions giving rise to a quarter of the adult. Professor Wilson, in a paper which he first gave as a lecture here at the Laboratory and published in 1893 under the title, "*Amphioxus* and the Mosaic Theory of Development," criticized this theory. Some person thought that this was the theory of Moses, the author of Pentateuch.

In 1892 Wilson published his "Cell Lineage of *Nereis*," one of the great classics of embryology. In 1890 and 1891 he did this research at this Laboratory. At the same time I was occupying the Johns Hopkins Table at the Fish Commission and was working on the Embryology of *Crepidula*, but had never met Wilson and knew nothing of his work on *Nereis*. Wilson heard of what I was doing and one Sunday morning he came over to see my drawings of *Crepidula* and to show me his of *Nereis*. I shall never forget our excitement. He had found, as I had, that there were three groups of cells that came off from the macromeres, that these gave rise to the whole of the ectoderm, and that the very next division of one of these macromeres gave rise to the mesoderm of the body. Here were two animals belonging to phyla as far apart as the annelids and the gastropods that gave rise to embryos in an exactly similar way. So we came to the conclusion that seemed necessary in those days, that there must be some phylogenetic relationship, that they came from common ancestors. If homologies are evidences of common descent, why should this not be true of cells as well as of organs? Thus arose at Woods Hole the cult of cell lineage, including besides Wilson and myself, Lillie, Mead, Holmes, Treadwell, Surface and a number of others who were finding individual blastomeres destined to give rise to particular parts of an animal.

But at the same time this was going on, the cult of "egg-shakers" became prominent at Woods Hole. This was about the same time that Driesch had broken eggs apart and found that each blastomere gave rise to an entire organism. Morgan was going about the laboratory shaking eggs in test-tubes. Morgan, Loeb, Child and others had all found, as Driesch did, that fragments gave rise to whole animals. So the question arose, who is telling the truth?

Apparently what was true of annelids and gastropods was not true of echinoderms; consequently I proposed the terms "determinate" and "indeterminate" cleavage for these two types. Later work shows that this is not a very useful or accurate distinction. The fact is that all eggs are partly determinate; at least we now know from the work of Hörstadius and others that this is true of echinoderms. In the case of the determinate eggs, they also have a certain amount of power of regulation. Consequently the difference is one of degree. One is relatively more regulative and the other is relatively more fixed. I found, for example, that ascidian eggs are highly mosaic, in consequence very definitely determinate, but in the case of *Amphioxus*, one-half of the egg will give rise to a whole animal, of half the size but beautifully developed. But if *Amphioxus* blastomeres are separated in the four-cell stage, right or left halves will give rise to a whole larva, but anterior or posterior halves will not; the anterior half-embryo would have the notochord and the neural plate but not the mesoderm. In the two-cell stage, each half has all the substances that are found in the other half and it needs only to close up the injured side by regulation to give rise to a whole embryo. The fact is that we are here dealing with a phenomenon that is wide spread throughout the animal kingdom, namely, regeneration, and the beliefs of the egg-shakers in this early period that because you could get a whole larva out of half or a quarter of an egg, that cells were therefore undifferentiated, had about as good a foundation as the view that because you can get a whole *Hydra* or *Planaria* or Annelid from a part of an animal, that therefore these animals are undifferentiated.

Driesch said that it was impossible to conceive of any machine that could be broken up in the three dimensions of space and the fragments remain capable of producing whole machines, and he claimed that the egg could be broken up and each part then grow into a whole animal; therefore he considered that the mechanistic theory of development was false. He did not sufficiently realize that living things are much more complex than a watch. In each cell there is a nucleus which is undifferentiated, which can in some cases remould the cytoplasm so as to bring about restoration of missing parts, because there is here a machine inside the machine, there is the outer machine which is the cytoplasm and there is the inner machine which is the nucleus, and as long as the nucleus is not broken up we may get regeneration.

Well, this all leads to the point toward which I have been aiming, namely that differentiation in development takes place in the cytoplasm. There

is no differentiation in the nucleus. In some way the nucleus does control differentiation in the cytoplasm; we know enough about what is happening in the egg to recognize some of the steps in the nuclear control of the cytoplasm.

In the case of many of the eggs you have been studying in this course you have found that during mitosis the nuclear membrane disappears and there is left in the cell body the relatively small chromosomes and a large amount of granular achromatic material which came out of the nucleus. The chromosomes then swell up and form chromosomal vesicles, and as the swelling goes on they get larger and larger, filling up with material taken from the cytoplasm until they form the large resting nucleus within which new chromosomes appear. At each division a large amount of achromatic material is set free into the cell body. I have followed this achromatic nuclear material in *Crepidula* up to the twenty-four cell stage and it always goes to that part of each cell which is nearest to the polar bodies; here it remains as granular material and ultimately cilia develop right over these granules. Thus we get differentiation from material which came from the nucleus and mixed with material in the cytoplasm. In this way differentiation occurs in the cytoplasm as a result of this "diastole" (swelling) of the nucleus and its "systole", when it breaks and releases material into the cell body. A certain amount of this interchange between nucleus and cytoplasm must take place in the absence of mitosis, but in mitosis you can see it most clearly. In this way differentiation in the cytoplasm comes under the control of the nucleus.

The localization of these different cytoplasmic substances in different cells and in different parts of the embryo is always a moving and in some cases a striking phenomenon. Eggs in which movements are rapid are most favorable. I don't know whether the *Styela* eggs are as good this year as they sometimes are. If you get animals which have a lot of orange color in the body, you will find that the eggs will have a large amount of that orange pigment and then you can see the sorts of things which are shown in this chart. In the early stages the pigment is scattered over the surface of the egg. Immediately after fertilization, this surface layer flows down to the point where the spermatozoon has entered. This material containing the yellow pigment then flows to the posterior side of the egg where it forms a crescent which gives rise to mesoderm. Another crescent of light gray material around the anterior side of the egg gives rise to the notochord and the neural plate. Between these crescents on the ventral side is the material of the ectoderm, on the dorsal side that of the endoderm. A similar mapping of the egg is found in *Amphioxus* and Amphibia. Regulation in the case of isolated blastomeres of these eggs depends to a certain extent, at least, upon the fluidity of their cytoplasm.

The late Dr. E. P. Lyon in 1906 used a centrifuge in some physiological work which he was doing here, and he tried it on sea urchin eggs and found that he could stratify the substances of the egg. This was a new instrument for studying the substances of eggs. Morgan and Lyon in the following year tried this out on eggs of the sea urchin and *Cumingia,* and they found that irrespective of where the pigment and yolk went, the embryo was perfectly formed. The conclusion was that these egg substances were not morphogenetic.

I centrifuged ascidian eggs and found that I could throw the yellow pigment, which is the lightest substance in the egg, to almost any point and still get normal development, so that this pigment is not morphogenetic. The muscles which normally contain this pigment will form when it is lacking; however when I centrifuged much harder, I found that I did dislocate materials that were morphogenetic, and then I could get animals with the muscles and chorda outside the body, with the ectoderm inside, with the eye spots inside or outside the body, indeed could bring about an abnormal distribution of the organs of the body by bringing about this abnormal distribution of morphogenetic substances in the unsegmented egg. When these substances of the ascidian egg are displaced by high centrifugal force, they produce what Virgil in the *Aeneid* called *membra disjecta,* organs scattered around in various ways out of position. I take this as evidence of the fact that we have morphogenetic substances in these eggs. No one has ever questioned the fact that the cytoplasm of the cells of the liver is different from that in the ganglion cells. The question is how early in the development can you find such differences, and I find that certain differences can be carried back to the one- or two-celled stage. *Amphioxus* eggs are just as plainly mapped-out as are those of ascidians There is here also a mesodermal crescent around the posterior half of the egg and a chorda-neural crescent around the anterior half, an endodermal area on the dorsal side and an ectodermal one on the ventral-anterior side. A similar mapping of the Amphibian egg is seen after fertilization in the "gray crescent" (chorda-neural) around the anterior side and a mesodermal crescent around the posterior. Yung has carried the mapping of these eggs much farther by locating on the egg the presumptive areas of all the principal organs of the embryo, but these areas are not generally marked out by visibly different kinds of oöplasm as is the case with the two crescents and the ectodermal and endodermal areas.

In 1901 Boveri found that soon after the fer-

tilization of the egg of the sea-urchin, *Strongylocentrotus*, a pigmented zone forms just below the equator. Below this pigmented zone is a clear area which gives rise to the four micromeres which form mesenchyme, the pigmented zone forms endoderm, while the area above the pigment becomes ectoderm. The three "germinal layers," ectoderm, endoderm, and mesoderm, are mapped out in this egg before cleavage begins.

The localization of morphogenetic substances in Annelids and Molluscs differs from that in Echinoderms, but in many respects resembles that in *Amphioxus* and Ascidians. The mesoderm comes chiefly from an area on the posterior side of the egg, while the ectoderm comes from the area around the animal pole and the endoderm from the vegetal portion of the egg.

In some of the hydro-medusae (e.g., *Linerges*) a concentric localization of substances in the egg is visible at an early stage, and in normal development the outer layer forms ectoderm, the inner, endoderm.

In all phyla there is a polar differentiation of the egg before fertilization, the upper or animal pole, at which the polar bodies form, giving rise later to the ectoderm, while the endoderm usually comes from materials of the opposite or vegetal pole. The axis connecting these poles bears a characteristic relation in different phyla to the chief axis of the developed animal. In some eggs bilateral symmetry or asymmetry is plainly visible before cleavage. And in a few favorable eggs even the location of the materials of particular organ-systems such as nervous system and sense organ, muscles and mesenchyme, notochord and endoderm are mapped out on the egg in areas of different colors or textures.

In conclusion, the egg is a living organism and its differentiations are the beginnings of the differentiations of the embryo and adult. Its polarity, symmetry and pattern give rise to those of the developed animal. This much of pre-formation of the future animal is present at the beginning of development. But the further details of development are the results of progressive differentiation resulting from the interaction of nucleus and cytoplasm. The nucleus is the seat of the inheritance genes, the cytoplasm of all the differentiations of development.

The differentiations of the cytoplasm begin at different stages and reach different degrees in different species and classes of animals, but always some differention begins before the fertilization of the egg. Therefore, the egg contributes more to development than the sperm does. The differentiations of the spermatozoon, i.e., acrosome, middle piece, tail, are lost after it enters the egg, but the differentiations of the egg persist. The spermatozoon reaches the end of its development before it is ready to enter the egg, but the egg reaches the end of its development only with the fully-formed animal. An egg can develop into an adult without the stimulus or contribution of a sperm, but no one has ever been able to get spermatozoon to develop apart from an egg. We derive more from our mothers than from our fathers. We are vertebrates because our mothers were vertebrates and produced eggs of the vertebrate pattern, but the color of our skin and eyes and hair and other features of later development are determined by the chromosomes from the sperm as well as those from the egg. The egg only undergoes embryonic differentiation.

I am still "the friend of the egg."

(This article is based upon a lecture delivered before the Embryology Class at the Marine Biological Laboratory on July 12.)

THE RECTIFYING PROPERTY OF THE GIANT AXON OF THE SQUID

DRS. RITA GUTTMAN AND KENNETH S. COLE
College of Physicians and Surgeons, Columbia University

The permeability of a living membrane to ions may be expected to be proportional to its electrical conductivity, since both of these properties depend upon the ease with which ions may pass through the membrane.

On passing an electric current through a living membrane, one finds that the electrical conductivity is greater under the cathode and less under the anode than normal. It has been assumed that the ion permeability is correspondingly increased under the cathode and decreased under the anode.

If the electrical conductivity or resistance (electrical conductivity is the reciprocal of electrical resistance) thus varies under the electrodes, the living membrane cannot be said to follow Ohm's Law. Ohm's Law states that the current is proportional to the voltage in a conductor over a wide range of voltages and currents, and the factor of proportionality may be termed the resistance, i.e.

$$\frac{E}{I} = R$$

where E represents electromotive force in volts, I represents current in amperes, and R is the resistance in ohms.

According to Ohm's Law, then, the resistance of a conductor should be the same under the elec-

trodes regardless of the magnitude of the current and regardless of the direction of the current but it has been found not to hold for the living membrane of the squid axon. Since the membrane of the squid axon permits current to pass more easily in one direction (outward), than in the other (inward), it is a rectifier rather than a pure resistance.

In two papers published in March, Cole, Baker and Curtis demonstrated rectification in the squid axon, using alternating current bridge methods and the needle electrode technique. These papers show that the previous failure of Cole and Hodgkin to observe rectification in this axon was due to the facts that only small currents had been used and that the effect under the anode was approximately equal and opposite to the effect under the cathode, thus neutralizing it.

In the work here reported, which was done at Woods Hole last summer, rectification was observed in the squid axon directly by means of a much simpler technique involving a direct current Wheatstone bridge. One end of the fiber was immersed in sea water, the interelectrode stretch was in oil and the other end of the fiber was injured by dipping it into KCl, so that a current travelling through the fiber passed across only one membrane. Thus neutralization of rectification under one electrode by rectification under the other was avoided.

The resistance of the fiber was measured during the passage of currents of varying magnitudes. The direction of the current was reversed at every reading thus minimizing polarization of the fiber membrane. Currents were sent in for short times only, and considerable and equal time intervals elapsed between readings, so that even though currents considerably above rheobase were often used, the condition of the fiber did not change materially over long periods of time. During a run there were frequent returns to a reference EMF value so that any shifting of resistance values with time and with temperature changes could be compensated for in the calculations.

The measuring technique used in these experiments has certain advantages. It is simple. It permits one to use an axon immediately after dissection. On the other hand, runs take about twenty minutes. The immediate problem is to speed up the measurements.

Simultaneous readings on excitability, resting potentials and rectification were made on single fibers as the fibers died. As the fiber dies not only does excitability disappear and the resting potential approach zero, but rectification, also, is gradually lost. The completely dead nerve fiber membrane acts like a pure resistance rather than a rectifier.

Progressive and partially reversible loss of rectification also accompanies narcotization of the nerve fiber with cocaine or veratrine sulphate.

The measurements show then that rectification is lost when the nerve fiber becomes inexcitable, when it dies, when it is anesthetized. Rectification thus seems to be associated somehow with the normal functioning of nerve.

Impedance and membrane potential measurements indicate that the rectifier effect is situated in the membrane, and it seems reasonable to assume that membrane conductance is a measure of ion permeability. These rectification measurements then indicate that there is an increased ion permeability under a cathode and a decreased ion permeability under an anode. Interpreting in a similar manner the results of Ebbecke obtained on frog nerve, we find large permeability changes at both anode and cathode, superimposed upon which there was a small permeability difference between anode and cathode regions. Blinks also studied rectification in the giant plant cell, *Valonia*.

In conclusion, rectification may assist in explaining various puzzling alternating current phenomena in nerve, such as change in membrane potential during the passage of an alternating current, the delay of excitation with alternating currents, alternating current block, alternating current break excitation, the time of rise of electrotonus being more rapid at the cathode than at the anode. However, an explanation of rectification, itself, is not as yet available. The explanation of rectification will probably also be an explanation of the mechanism of the ion permeability of the membrane.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 8.)

THE DISTRIBUTION AND DEVELOPMENT OF THE MELANOPHORE HORMONE IN THE PITUITARY OF THE CHICK

DR. H. RAHN
Instructor in Zoology, University of Wyoming

The intermedin hormone or the melanophore dispersing principle is commonly derived from the pars intermedia of the typical vertebrate pituitary gland. In the birds, however, this hormone presents a peculiar problem, because a structural pars intermedia is absent. Furthermore, its effect upon the bird melanophore or any other function it may have in the bird or mammal has not been demonstrated. However, this hormone is present in considerable quantities in the chicken pituitary gland as tested by its pigment dispersing qualities in lower vertebrates. It is therefore not to be regarded as a hormone that has lost its function in the warm blooded animals, but very probably

plays an entirely different role which still awaits discovery. Such a change in hormone function during the course of phylogenetic development must be kept in mind, since, for example, the prolactin hormone of the pituitary has been demonstrated to exert widely different effects in the various vertebrate groups. In amphibia, this hormone seems to induce the migration urge of the land newt to seek a water environment, in the bird it acts directly on the crop gland of the pigeon, and in the mammal it is concerned with milk secretion in the mammary gland.

The more immediate attack upon the melanophore hormone problem as presented here may be outlined as follows: (1) where is this hormone found in the absence of a structural pars intermedia? and (2) does a study of the formation of this hormone during development give us any possible clue to its function?

Morphology of the bird pituitary. At the time this work was begun the presence or absence of a pars intermedia in the bird was still debated. A detailed cytological study of the development of the chicken pituitary gland revealed, however, that at no time during ontogeny or later on could such a structure be demonstrated. Furthermore there did not exist any connection between the infundibular process of the diencephalon and the glandular portion, the pars buccalis. In the typical vertebrate there is an intimate connection between these two components contributed by the presence of a pars intermedia. Since the case of the chicken might possibly represent a specialized type (as has been found in some mammals which have no pars intermedia) a comparative study was undertaken to see how widespread this morphological peculiarity is. Altogether 18 species were studied, representing 12 families and five orders. They all revealed a pituitary construction identical in pattern to the one found in the chicken.

Distribution of the hormone. The infundibular process had for a long time been suspected in higher forms to yield at least part of the melanophore hormone, since it was never possible to separate it completely from the closely applied pars intermedia. In the chicken this separation is normally present and tests revealed that no melanophore hormone could be found in this nervous component. This then suggested that the pars anterior is entirely responsible for hormone production and it became of interest to find out just where it was found within the glandular component. From a phylogenetic standpoint one might expect it to reside in the region nearest the infundibular process. Quantitative assays, however, revealed that it is found in all regions, but is 20 times more concentrated in the region furthest removed from the nervous lobe.

After ascertaining the distribution of this hormone within the gland, the next problem concerned itself with the quantitative assay of the melanophore hormone during ontogeny. Preliminary tests and observations on amphibian larvae revealed that not only is hormone present in the pituitary, but also functioning in melanophore dispersion at an extremely early age. Histological sections revealed a very much undifferentiated pituitary gland. This rather unusual situation prompted a similar and more detailed investigation in the chick pituitary, since in this form the cytological differentiation had been worked out.

Assay Method. For the quantitative determination of the melanophore hormone the *Anolis* lizard was employed. This animal exhibits most striking color changes, from a bright green to a dark brown. The metachrosis is produced by the concentration and dispersion of pigment in the melanophores and is regulated by the melanophore hormone of the pituitary gland. When the pituitary complex is removed the animal is permanently green and becomes very sensitive to this hormone. In fact 1/10,000 of mg of dried bird pituitary tissue will evoke a darkening response.

The degree of darkening is proportional to the hormone concentration injected into such a hypophysectomized animal and four successive stages of darkening from bright green to dark brown have been assigned arbitrary values of stages 1-4. For the actual test a known fraction of the pituitary gland is injected into ten operated animals and their average color response recorded as 1, 2, 3, or 4. From several such determinations the exact concentration can be calculated which will evoke precisely a color response of stage 1. This concentration of hormone is then designated as 1 *A*(nolis) *U*(nit). (*Anat. Rec.*, 76, 157).

Hormone genesis. By this method the melanophore hormone was determined for various age groups. The first qualitative test was obtained on the fifth day of incubation, that is five days before the first visible signs of cytological differentiation in the pars anterior. From the seventh day on enough hormone for quantitative assays could be found. The general results throughout development are tabulated below. The adult gland contains about 900 A.U. which means that one gland has enough melanophore hormone to darken 900 hypophysectomized lizards to stage 1. It is of interest to note the second column which shows the A.U. per unit weight of tissue. It may be

Age in days	A.U. per gland	A.U. per 100 γ of tissue
5	present	
7	0.2	—
9	0.8	—
12	5.0	9
15	11.0	13
21 (hatching)	90.0	60
49	166.0	56
adult	909.0	77

seen that the concentration of the hormone per unit mass does not increase after hatching. All apparent increase can be accounted for by the growth of the gland. It seems to suggest that cell for cell the pituitary reaches its peak of production at the time of hatching and maintains it there.

But of even greater interest is the early appearance of the melanophore hormone during development. From analagous observations in the amphibian it may very possibly be in circulation by the sixth day of incubation. Other hormones which function early in development is the thyroid stimulating factor of the pituitary, but it appears on the 11th day of incubation and the gonadotropic factors are probably not needed until later in development. Thus the extremely early appearance of the melanophore hormone suggests that its stimulation may be a very general, possibly a metabolic one.

Another aspect of this problem concerns itself with the interpretation of cytological differentiation. Here we have evidence of hormone formation and, at least in the amphibian, of hormone release long before we can detect or suspect such changes by histological methods. Such circumstances must question the reliability of cytological interpretation of glandular function in embryonic tissues.

(This work was done in collaboration with Dr. Painter, Dr. Kleinholz and Mr. Drager. This article is based upon a seminar report presented at the Marine Biological Laboratory on July 22.)

THE ORGANIZATION OF THE MELANOPHORE SYSTEM IN BONY FISHES

(Continued from page 81)

upward therefrom, some enters the eye, passes obliquely upward through that structure and thus reaches the dorsal retina. This reflected light is the significant light in exciting blanching as demonstrated in several other fishes than the catfish by Sumner, Hogben, and especially by Butcher. That this is so can be shown in several ways. When a fish is illuminated by light which is thrown exclusively from below so that nothing in the eye except the dorsal retina is effectively illuminated, the animal will blanch. If the dorsal retina is first destroyed and the eye is then illuminated from below, no blanching will occur. Further, if the eye is rotated on its axis through 180 degrees so that the dorsal retina comes to lie ventrally, blanching occurs only when light reaches this transposed dorsal organ. Thus blanching occurs only when the dorsal retina is illuminated and is best seen when this part of the eye is the exclusive recipient of light. Blanching, however, will take place when light reaches the ventral retina as well as the dorsal one but the change is not so complete as when this agent impinges exclusively on the dorsal retina.

From the dorsal retina nerve tracts pass through the central nervous organs and over the autonomic system from which they finally emerge as adrenergic fibers whose terminals are in close proximity to the melanophores. The adrenaline discharged at these terminals (Chang, Hsieh and Lu; and Parker) induces the concentration of melanophore pigment whereby the fish is made to blanch. Such a system of fibers may be designated as a retino-adrenergic arc.

The blood of a fully pale catfish when injected into other catfishes either pale or dark will cause no change in their tints. Hence the blood of such fishes must be devoid of any physiological traces of the melanophore-expanding principle of the pituitary gland, intermedine; in other words the activity of this gland appears to be inhibited in the pale state of the fish. It is therefore probable that the dorsal retina not only excites impulses to blanching over the retino-adrenergic arc but also other impulses that check intermedine. The nerve tracts from the dorsal retina to the pituitary gland over which these impulses pass, if this is a nervous operation, may be called collectively the retino-pituitary inhibition arc.

The dark phase of the catfish involves the two receptors, the ventral retina and the skin, and is best seen when the fish is on an illuminated black background. The action of the ventral retina is most conveniently studied in hypophysectomized fishes. The eyes of such a fish on a black background illuminated from above receive light only on the ventral retina. This light is light direct from the source of illumination. In this case there is no reflected light to pass to the dorsal retina, for such light as would be reflected is absorbed by the black background. The ventral retina thus excited gives rise to nerve impulses that pass through the central nervous organs and out over autonomic tracts to the melanophores. These tracts contain the cholinergic fibers which discharge acetylcholine and thus induce a dispersion of melanophore pigment. This dispersion is, however, only about half that of which the melanophore is capable as was first shown by Osborn. It may be completed by injecting intermedine into the fish. The tracts that reach from the ventral retina to the melanophores may be designated the retino-cholinergic arc.

The second receptor concerned with the darkening of the catfish is the skin. This fact can best be demonstrated in eyeless fishes. Pale catfishes, catfishes of intermediate tint, and dark catfishes if completely enucleated and immediately put into perfect darkness, retain their original tint without change for many days. When brought out of darkness and into the light they quickly become

(Continued on page 93)

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris I. Gorokhoff and Judy Woodring.

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M.B.L. CLUB NOTES

The second "Mixer" of the season will be held this evening, beginning at 8:30 P. M. Designed primarily to help the newly arrived students in invertebrate zoology to meet other biologists at Woods Hole, the Mixer will consist of a social hour, followed by dancing in which the Paul Jones and other dances will help to "mix" people. An unusual feature of the decorations will be aquaria with living animals generously provided by the Supply Department. Refreshments will be served. Name cards, with students' names lettered on them, are being made by a committee consisting of Mary Chamberlain, Dr. Perry Gilbert and Dr. James Goldinger.

The Annual Meeting of the M.B.L. Club was held Monday evening at 7 P. M. Mr. C. L. Claff was reelected President of the Club; Dr. Sears Crowell, who had been Secretary-Treasurer, was made Vice-President; and Mrs. Elsa Keil Sichel was elected Secretary-Treasurer. Mr. F. M. MacNaught was elected Trustee on behalf of the Club, and Dr. E. R. Clark was made Trustee on behalf of the Laboratory. Reports were presented for the House Committee by Mrs. Karl Wilbur, its chairman, and for the Social Committee by Mrs. T. H. Bullock. The Constitution of the Club was amended to provide for a sinking fund for repairs to the Club House.

The "Poverty Dance" held at the Club House last Saturday night was thoroughly enjoyed and well attended. Persons attending were dressed in old and bizarre costumes, and prizes were awarded for the best outfits; an entertainment program was presented which was followed by square dancing. The committee to judge the costumes, consisting of Dr. W. W. Ballard, Dr. James Goldinger, Miss Mary Chamberlain and Mr. Arthur Woodward, awarded prizes to the following persons: Mrs. Sears Crowell, for the most beautiful costume, Mrs. Karl Wilbur, for the most unusual costume, with honorable mention to L. Gilman, and Mr. C. L. Claff, for the "stupidest" costume.

Entertainment, under the direction of J. P. Trinkaus, was presented with Teru Hayashi as master of ceremonies. The program opened with songs by the "Embryology Trio," composed of Tom Morgan, tenor, Irving Plough, baritone, and Jack Gross, bass. Then came the "Three Blind Lice," consisting of Hermann Rahn, Mrs. Jeanette Renshaw, and Bob Knapp; songs by Arlene Mothes; a jitterbug dance by Dick and Jane Henry; and a skit in which Frank Hartman, Bob Harrison and J. P. Trinkaus took part.

The Decorations Committee consisted of Miss Mary Chamberlain, Mrs. C. L. Claff, Mrs. Laurence Hobson, Miss Marilyn Bosworth, and Miss Marion Davis. The refreshments were in charge of Mrs. Wilbur.

Folk dancing is being revived at the Club this year under the direction of Dr. S. E. Pond. The first meeting was held Wednesday night. Dancing is scheduled to take place on Wednesday evenings at 7:00 P. M.

Play for the annual ping-pong tournament at the Club will start on Monday, August 11. All persons interested should see Teru Hayashi for details. The ping pong table will be available until that date for training and practice.

The program for the weekly phonograph record concert at the M.B.L. Club next Monday is as follows: Resnichek, "Overture to Donna Diana"; Schumann, "Concerto in A for Piano and Orchestra"; intermission; Mozart, "Symphony No. 40"; Sibelius, "Finlandia."

Dr. Henry B. Bigelow, curator of oceanography at the Museum of Comparative Zoology at Harvard University, was awarded the honorary degree of Doctor of Science at the commencement exercises at Yale University on June 18. He was formerly director of the Woods Hole Oceanographic Institution and is now one of its trustees.

Dr. Robert Cushman Murphy, curator of oceanographic birds at the American Museum of Natural History, received the honorary degree of Doctor of Science from Brown University on June 16. Dr. Murphy lectured at the Marine Biological Laboratory in 1937 on "The Gates of the Antarctic."

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
July 25	5:14	5:27
July 26	5:57	6:12
July 27	6:40	6:58
July 28	7:25	7:48
July 29	8:13	8:40
July 30	9:04	9:36
July 31	9:58	10:36
August 1	10:55	11:38

In each case the current changes approximately six hours later and runs from the Sound to the Bay.

ITEMS OF INTEREST

The formal classwork for students in invertebrate zoology at the Marine Biological Laboratory began on Friday morning when Dr. Waterman lectured on protozoa. They registered and had their desks assigned to them during the previous afternoon. On Thursday evening the director of the course, Dr. T. Hume Bissonnette, met the class to give them an introductory talk concerning matters of general interest.

Dr. Perry W. Gilbert, instructor in zoology at Cornell University, has replaced Dr. Samuel A. Matthews as junior instructor. The laboratory assistant this year is Dr. J. W. Bowen, assistant professor of zoology at the University of North Carolina.

Fifty-five students—the maximum number that can be accommodated—are taking the course; many more made application for admission. The class remains in session through August 30.

DR. OTTO GLASER, professor of biology on the E. S. Harkness Foundation at Amherst College, has been appointed acting president of Amherst College in the absence of the president.

DR. DAVID R. GODDARD, assistant professor of botany at the University of Rochester, and instructor in the Botany course at the Marine Biological Laboratory, has been promoted to an associate professorship at the University.

DR. JAMES D. HARDY, formerly research associate of the Russell Sage Institute and now on active duty in the U. S. Navy, has been appointed assistant professor of physiology at Cornell University Medical College.

DR. J. S. RANKIN, JR., who has been instructor in biology at Amherst, has been appointed to an assistant professorship of biology at the University of Washington. He is teaching in the invertebrate course at the Marine Biological Laboratory.

DR. T. H. BULLOCK has received a Rockefeller Foundation Fellowship for the coming academic year, and will work in the department of experimental neurology at Yale University. Last year he was at Yale on a Sterling Fellowship in zoology.

DR. GEORGE E. COGHILL, member of the board of advisors of the Wistar Institute, died in Gainesville, Florida, on Wednesday at the age of 69. He had been professor of comparative anatomy at the Wistar Institute from 1925 to 1935, and was managing editor of *The Journal of Comparative Neurology* from 1927 to 1933.

DR. AND MRS. CHARLES PACKARD will be at home to members of the Laboratory tomorrow from four-thirty to six o'clock.

MR. RAY WATTERSON will marry Miss Evelyn Goddard next week in Boston. After the ceremonies, the couple will return to Woods Hole, where Mr. Watterson will continue his work. They will be at the Johns Hopkins University during the next academic year.

DR. AND MRS. HERBERT H. BROWN are spending a few days at the Bureau of Fisheries Residence as the guests of Dr. P. S. Galtsoff. Dr. Brown has just completed an appointment as director of the sponge fishery investigations of the Bahama Islands. He is a member of the staff of the British Colonial Fishery Service, and is now awaiting instructions.

MR. H. I. ANDERSON, business manager of *Biological Abstracts*, visited Woods Hole for two days early this week.

DR. AND MRS. P. B. ARMSTRONG have recently become the parents of a son. Dr. Armstrong will give the Friday evening lecture on August 8 and will work at the Laboratory for several weeks.

The Embryology course of the Marine Biological Laboratory ended on Tuesday, the Physiology course on Wednesday and the Botany course ends today.

The *Atlantis*, after returning from a trip under the direction of Dr. Henry C. Stetson, sailed on July 21 to Brooklyn, where she will be in drydock for several days. The *Anton Dohrn* is still out on a trip under the direction of Mr. F. Fuglister.

At the weekly staff meeting at the Woods Hole Oceanographic Institution on Thursday evening, Fred B. Phleger, Jr., of Amherst College spoke on "The Use of Foraminifera in Determining Marine Sediments."

The Yalden Sundial, on the shore opposite the Marine Biological Laboratory, was damaged recently by vandals who pried off the bronze plate carrying the chart of instructions. The Laboratory has taken steps to replace the plate.

The J. B. Lippincott Company is holding an exhibit of books at the Old Lecture Hall; the Clay-Adams Company closes its exhibit there today. The Bausch and Lomb Optical Company is continuing its exhibit at the Coast Guard Canteen. The Blakiston and Saunders Companies are exhibiting their publications in the Lobby of the Brick Building.

PHYSIOLOGY CLASS NOTES

We have become so micro-minded lately that even the news this week seems to be all on the micro side, quantitatively as well as qualitatively. What with a micro Van Slyke (of Hal Gordon's design), microanalyses, and other things micro going on, we bid fair to present a micro column.

On Monday Dr. S. E. Hill spoke on "The Action Current of *Nitella*." The subject of Dr. E. S. G. Barrón's lecture on Tuesday was "Oxidation-Reduction Systems in Cellular Respiration." Dr. D. Wrinch talked on "Protein Structure" on Wednesday.

With the breakage derby finished we have turned from glassware to records in our destructive endeavors, and have succeeded in shattering a long victory record held by the crew in baseball. The score: 7-4. A mention of stellar performances would be a roster of the entire team, including the faculty representatives, Drs. Kempton and Fisher.

Total immersion came, as it must to all kibitzers, last week to one Jasper P. Trinkaus. On the occasion of the class photograph, his fourth of July left-overs spoiled a couple of fine poses. His subsequent entrance into Eel Pond was unfortunately not photographed for posterity. In a way it was a Pyrrhic victory, for our own John Gregg was dragged in, too. The Philip Morris representative, taking advantage of the occasion, passed cigarettes out among the crowd on the dock, making the incident even more worthwhile.

You have probably already glanced over our scientific looking little group of physiologists on the inside front cover and now realize just what a physiology student should look like. Special mention should be made of Dr. Fisher, who appears to be expecting something from heaven.

The high tide of the summer occurred on Wednesday when the course was officially over. It was caused by the salt tears of all the physiologists who, in deep sorrow, packed up and checked out, homeward bound, having finally reached the end of a much enjoyed and long-to-be remembered five weeks. —*The ex-Mr. and Mrs. J. B. V. S.*

BOTANY CLASS NOTES

If you've got imagination and would like to have some fun,
Just open all the stops and cocks and let it wildly run . . .
We'll show you whom the Hole will miss when this year's course is done.

Sam, Sam, the algology man,
Plays hookey from lab whenever he can;
He winks at the women and pitches for crew.
What kind of a guy is he? We wish we knew!

Nancy, Nancy, with ideas romancy,
Dodging the romeos flocking her door;
Though she may have a cock of the head that's quite pert,
We really can't picture this girl as a flirt.

Can you see Robert Muir without his curly hair,
Sans cigarette holder and supercilious air?
Not resting on a bucket in the middle of the drink,
Or collecting his algae at home in the sink?

With a falsetto guffaw, algologist Abbott
Wouldn't be our chuckling "Peter Rabbit",
And if, without puffing, he kept up with Taylor,
At the end of a field trip, he'd be even paler.

Without "Tell me more" eyes on a Saturday nite,
With her windblown hair always combed just right,
You wouldn't know Babs of the red peely nose,
Who daily for "weeds" to the drugstore goes.

Imagine gruff Monti without his sly smile,
Not being dead serious and ribbing the while,
And telling the girls that he will dare 'em,
To leave the lab and join his harem.

Picture Connie Stanton with tresses raven black,
Deigning—at Muir's silly cracks—to even answer back,
Or robbed of personality, without that sparkling vim,
Not dashing from the lab each day to follow some fool whim.

Picture Felix keeping quiet and not forever talking,
See her going on field trips not complaining of the walking,
Picture her without a question, silent and demure,
Managing remarks from Monti to patiently endure.

Imagine Ruth Franz not a quiet, sweet, girl,
Can you see her going round in a wild, frantic whirl?
Rebelling 'gainst orders to "Go get my jacket!"
Can you picture her ever making a racket?

Imagine Jean Enzenbacher far, far less serious,
Demanding some scissors with voice most imperious.
Picture the girl never catching a cold,
Can you see her frivolous or terribly bold?

If Axel has shoes on, it's something quite serious,
Can you picture him coming with shoes on the "Nereis"?
Without his hip boots and his swordfisher's cap,
Or steering a course with the aid of a map?

Can you picture Robert Thorne without his air-conditioned pants,
Not starting water fights or out a-heckling, say, Ruth Franz?
Or making accusations of imaginations rare,
When the girls find parts of algae that he didn't think were there?

Picture Dr. Smith, the tall, a mere five feet, no more,
Not beaming with that friendly grin, but getting really sore;
Or picture him within the mire of lowly family strife,
'Cause work in lab had made him late to luncheon with his wife.

Imagine Bob without his Betty at the Mess for lunch,
And picture Williams not the clown in any sort of bunch;
And picture him a chemist with organic chem down cold—

Without alginic acid, he's not the Bob of old.

Imagine anything collectable—by cracky,
Not being collected by our Babe Ruth Jackie;
Without flowers in hair or starting a game,
It's surely not Waldron who's so all-fired insane.

Imagine some of us going to the bother,
Of fearing the wrath of the tolerant Father,
And doing our swearing completely in Spanish,
Lest the culprits from Botany lab he should banish.

Picture Augusta without the Victoria,
Harriette, Antonia, and Leuchs in there, too;
Not puffing her corncob each nite after tea,
Wearing hair that's unkempt and expression that's blue.

If you could do as we've suggested and imagine this strange crew,
You'll be as glad as we are sad that this Botany course is thru.

—*J. W. and C. S.*

EMBRYOLOGY CLASS NOTES

On Wednesday, July 16th, Dr. Hamburger considered the Molluscan larval stages omitting atypical *Loligo* studied earlier in the course for attention to the more typical members, *Crepidula* and *Teredo*.

On Thursday the speaker's table had its heaviest use of any day in the course for it supported the references and materials for three solidly packed lectures for three sure scientists. In the morning, Dr. Hamburger started the series with a review of past and recent experimental work in the embryology of the Annelida and the Mollusca. In the afternoon Dr. Walter Landganer's lecture on disproportionate dwarfism in the chicken came as a surprise to the class.

In the evening Dr. Harold Plough lectured on "Genes in Development" to an extra-large audience. Along with many, many other facts Dr. Plough made it clear that the time of gene action varies.

Dr. Ballard took over the course on Friday for the presentation of the final materials of the course. This day it was wet Coelenterates and they balanced very well with much dry humor.

Saturday started out to be a disappointing day for the embryologists for their scheduled towing was cancelled due to high winds from an unfavorable direction. Yet this was soon forgotten when examination of tows obtained earlier in the day was begun. Every marine biologist, manual, and textbook in sight was practically worn out in two hours, so anxious was the class to learn about oddities they discovered.

Dr. Caswell Grave gave the last formal lecture of the course on Tuesday morning. His subject was metamorphorsis in the Ascidians. Throughout the lecture Dr. Grave said everything to nullify his preliminary slogan, "Work with Ascidians and be lonesome." Conditions favoring, I am sure that Dr. Grave would have had thirty-seven coworkers at the end of his lecture. By afternoon the students had begun to depart. Happy for having lived five such edifying weeks, sad for having to part from such good friends, the students of the 1941 Embryology Class went their scattered ways. —*E. R.*

THE ORGANIZATION OF THE MELANOPHORE SYSTEM IN BONY FISHES

(Continued from page 89)

coal-black. The blood from these fishes when injected into pale fishes will darken the recipients, for it contains intermedine. Apparently the photoreceptors of the catfish skin when excited by light give out impulses that pass through the central nervous organs to the pituitary gland which is thereby excited to discharge intermedine. This in turn is carried by the blood to the melanophores which respond by complete pigment dispersion. If the brain of a catfish is completely transected immediately in front of the cerebellum, the eyes and the pituitary gland are left intact on the anterior part of the central nervous fragment and the whole skin innervation remains unaltered on the posterior part. Catfishes that have undergone this operation show no obvious color changes, which indicates two important conclusions; first, that the eyes of this fish are not concerned with exciting the discharge of intermedine from the pituitary gland, and, second, that the darkening of the skin in the catfish is not a spinal-cord reflex. The tracts by which the skin photoreceptors in the catfish are connected with the pituitary gland and the blood courses by which the intermedine from this gland is carried to the melanophores may be called collectively the dermopituitary arc.

Of the bony fishes whose color changes have been studied within the last few years the following appear to conform in general to the type of chromatic organization described for the catfish in so far as they possess both adrenergic and cholinergic fibers and intermedine: angelfish (Tomita), eel (Waring), snakefish (Chang, Hsieh and Lu), Japanese catfish (Matsushita), and stickleback (Hogben and Landgrebe). However, in none of these instances is it known that the skin acts as a receptor in the way that it does in the catfish. Certainly in the killifish and probably in flatfishes (Osborn) adrenergic and cholinergic fibers are present, but intermedine appears to play a very subordinate part in these forms. Further study will probably show that the chromatic systems of different bony fishes are specifically individual rather than that they conform to a single type of organization.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 22.)

SUPPLEMENTARY DIRECTORY FOR 1941

ADDITIONAL INVESTIGATORS

Aquila, (Sister) M. grad. biol. Villanova. Rock 3.
Beck, L. V. instr. phys. Hahnemann. lib.
Boche, R. D. instr. zool. Pennsylvania. Br 221. D 112-A.
Bodian, D. asst. prof. anat. Western Reserve Med. lib.
Bronfenbrenner, J. prof. bact. & immun. Washington Med. (St. Louis). Br 305.
Brown, D. E. S. prof. phys. New York. Br 310.
Calkins, G. N. prof. proto. Columbia. Br 331.
Castelmano, Gina zool. Minnesota. lib.
Chambers, E. New York Med. Br 343.
Chambers, R. prof. biol. New York. Br 328.
Clark, Eleanor L. assoc. anat. Pennsylvania Med. Br 117.
Duncan, G. W. fel. surg. Hopkins. Br 328.
Erlanger, Margaret instr. West Virginia. Br 312.
Finkel, A. J. grad. asst. zool. Chicago. Br 322.
Garner, H. res. asst. zool. Chicago. Br 332. Ka 22.
Gelback, Elizabeth L. asst. proto. Yale. Br 323.
Genevieve, (Sister) Mary grad. biol. Villanova. Rock 3.
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THE COLLECTING NET

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EFFECT OF SEA WATER ON THE RADIOSENSITIVITY OF *ARBACIA* SPERM

DRS. T. C. EVANS AND J. C. SLAUGHTER

Departments of Radiology and Zoology, State University of Iowa

In the course of an investigation of the effects of roentgen radiation on the oxygen consumption of *Arbacia* sperm it was noted that dilute suspensions of sperm were injured more by the radiation than were sperm irradiated in concentrated suspensions. This observation was checked by irradiating sperm in different concentrations of sea water and later determining the fertility of each lot. This was done by adding the same amounts of control and irradiated sperm to similar lots of eggs. The percentage fertilization was taken as the number of eggs which raised fertilization membranes per hundred counted. In a series of five experiments consistent results were obtained which indicated that as more sea water was added to the sperm their radiosensitivity increased. The results of one of these experiments are shown in Table I.

It will be noted also (*Continued on page* 113)

M. B. L. Calendar

TUESDAY, August 5, 8:00 P. M.

Seminar: Dr. Victor Schechter: "Aging Phenomena, and Factors Influencing the Longevity of Mactra Eggs."

Dr. Frederick S. Philips: "Comparison of Regional Respiratory Rates of the Chick Embryo during Early Stages of Development."

Dr. Matilda M. Brooks: "Further Interpretations of the Effects of CO and CN on Oxidations in Living Cells."

FRIDAY, August 8, 8:00 P. M.

Dr. P. B. Armstrong: "Function in the Developing Gastro-Intestinal Tract of Amblystoma punctatum in Relation to Embryonic Determination and Differentiation."

STRUCTURE OF THE RED CELL IN THE LIGHT OF SHAPE TRANSFORMATIONS

DR. ERIC PONDER

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Since the circular, biconcave form of the mammalian red cell was first correctly described by Hodgkin and J. J. Lister in 1827 (over 150 years after the discovery of the cell by Swammerdam and later by Leeuwenhoek) there has been an almost continuous speculation as to the reason why this special shape should be assumed by bodies floating freely in a liquid. Generally speaking, the theories which have been advanced have been of two kinds: those which attribute the biconcave shape to forces or structures in the interior, and those which attribute it to forces or structures at the surface.

Into the first category falls the "gelatin lozenge" theory of Rollett (1862) "who conceives that a stroma or matrix enters into the structure of the colorless elastic extensible substance of the red corpuscle, and that to this the form and the peculiar physical properties of the corpuscles is due. It is supposed that the coloring matter

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AIRPLANE VIEW OF WOODS HOLE

with Juniper Point, the Buoy Yard, and Little Harbor in the immediate foreground. The biological laboratories are in the center of the upper right-hand quarter of the picture.

can be separated from the stroma without causing the latter to lose its essential characters" (quotation from Norris, 1882; Thudicum, writing at the same time, goes further, and defines the stroma as a chemical skeleton, with which the hemoglobin is combined, an idea which has been revived by Lepeschkin, Adams, and others). Gough and Teitel-Bernard have suggested that molecules in the cell interior, and particularly hemoglobin molecules, repel one another more in one axis than in another, and so give rise to the discoidal shape in a cell with a fluid interior, but this hypothesis cannot be held in view of the fact that hemoglobin-free ghosts are discoidal.

The second point of view is by far the older, and although it is associated with the names of Schwann and of Hewson, it was originally expressed by Bidloo in 1685 and by Wells in 1797. It was emphatically defended, particularly against the view of Rollett, by Schafer (1891), whose description of the cells as "vesicular bodies possessing an external envelope enclosing a fluid interior" has come to be known as the "balloon theory". In 1882, Norris was impressed, as several others have been (Rice, 1914, Gough, 1924), by the similarity of shape between red cells and the myelin forms of lecithin. The latter are often circular discs about 5μ to 10μ in diameter, and are dumb-bell or ring shaped in cross section. They are apparently formed by physical forces at the interfaces between the droplets and the fluid surrounding them, and Norris suggested that the biconcave shape of the mammalian red cell is brought about in a similar way. "The remarkable properties displayed by myelin at once relieve us from the necessity of considering that one liquid or solution submerged in another must inevitably take on globular or spherical state. The fact is, the substance appears to represent the extending or spreading-out tendency, as opposed to the gathering-up or sphere-forming property. The biconcave and the annular forms seem to be related to a kind of balancing of these two properties. . . . I consider, too, that the form which these bodies assume is as dependent on the constitution of the liquid in which they are submerged as on their own. . . . It would therefore seem that the biconcave form is to be regarded as an arrested annular form. *This annulating property belongs to the corpuscle as a substance* (italics mine) for it occurs in fused masses of corpuscles, in single corpuscles, and in fractional parts of them" (Norris). Or, as Gough puts it, there are two sets of forces operating, the first of which tends to produce contraction of the surface and the spherical form, while the second tends to bring about expansion and a very flattened form; balanced against each other, the two sets of forces maintain the discoidal form. It is not difficult to see how mutual repulsion between molecules in the surface layers might arise, for hydrocarbons with polar groups directed towards the water (as in lecithin) might exhibit repulsion of each other.

Some idea of the forces involved may be obtained by drawing a cross section of the red cell to scale, finding the two principal radii of curvature ρ_1 and ρ_2 at each point, and computing the pressure P which would have to be applied to keep a homogeneous cell membrane, with tension T, in hydrostatic equilibrium:

$$P = T(1/\rho_1 + 1/\rho_2).$$

It appears that we have to have a pressure directed outwards over the equatorial regions of the cell, and a smaller pressure directed inwards over the biconcavities, if we are to arrive at the shape in this way. The idea that such pressures really exist is, of course, untenable, but the "outward pressure over the equatorial regions" is the same thing as Gough's "expansive force". What really happens is probably a variation in the tensions from point to point along the membrane, and this is the same as saying that the membrane is not molecularly homogeneous, or has a "liquid crystal" structure.

In 1926 (Ponder, 1933) I tried to put this attractive idea into mathematical form, with a suggestive, if disappointing, result. I evaluated the radii of curvature for the red cell of man, and, on the assumption that the tensions in the membrane are equal at all points, obtained a series of values for the pressures which would maintain the shape; if, as is probably the case, the pressures are equal and the tensions vary, the true relations are derivable from the same sets of values, and any theory proposed for the shape of the red cell must be one which gives these relations, at least substantially. I then tried to find the shape of a body of given volume, given area (not necessarily the smallest area for the volume, for then the body would be spherical) and with the smoothest surface, and arrived at a form suggestive of that of the red cell; the derivation, however, was faulty in that it dealt with a mathematical surface rather than with a surface with real elastic properties, but the result was interesting in showing how a problem of this kind might be approached, and

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what a formulation of Norris' theory might involve.

The first problem, then, is to decide whether the form of the red cell is due to forces or structures in the interior, or to forces or structures at the surface. Microscopical observation, either with direct illumination, with the dark field, with ultraviolet light, or with the electron microscope, tells us little one way or the other about the existence of a stroma, and evidence from microdissection studies is equally inconclusive; sometimes the red cell disappears altogether when punctured, but sometimes there is a mass left behind which can be teased into shreds. This may be a product of gelation, for the red cell has been shown to contain proteins other than hemoglobin, e.g., the stromatin of Jorpes (1932) and of Boehm (1935), which is capable of forming a remarkably rigid gel in low concentrations. Turning to the surface, we find a distinction between the cell "wall" or "envelope" and the cell "membrane", the former being at least thick enough to be visible (0.05μ), while the thickness of the latter has been put at anywhere from 30 A. to 300 A. or more. We have no indication as to how forces responsible for the shape of the cell might arise at such a surface, and both Rollett's theory and Norris' theory demand some kind of special structure (a "stroma", or a special molecular configuration at the surface), and we do not have much direct evidence that such a structure exists. We may accordingly turn to the shape transformations of the red cell, and see what they tell us.

1. Disc-sphere transformations.

As hitherto described, these are five in number, and the study of each brings out some special point which has a bearing on the subject of red cell shape.

1. The spherical form between two surfaces. Mammalian red cells, suspended in 1 p.c. NaCl or any of the ordinary physiological salines, and examined in a hanging or uncovered drop, are discoidal although often crenated; at all events, they are not spherical. If the same cells are covered with a coverglass so that the amount of fluid between the two glass surfaces is very small, the cells become smooth spheres of the same volume as were the original discs. If the two surfaces are in close contact, the change is very rapid, but by constructing a wedge-shaped chamber it can be seen that the transformation involves intermediate forms; the discs first crenate, the crenations becoming progressively finer until a prickly form, the "thorn-apple" form, is assumed; the crenations then become finer still and are ultimately smoothed out, so that a glistening sphere results. If a small amount of serum or plasma is run under the coverglass, the spheres become crenated, the crenations become larger, and the discoidal form re-appears; these re-converted discs may not be as perfect in shape as the original cells, and they are unusually sticky. Millar (1925) describes them as being mottled when seen with the dark field.

Since the original description of the spherical form by Hamburger in 1895, there has been much speculation as to the cause of this transformation, but in 1940 Furchgott showed that it is due to (a) an increase in pH produced by diffusion of alkali from the glass surfaces, and (b) the removal from the cells of an "anti-sphering substance", which he and I later showed to be the carbohydrate-poor fraction of serum albumin, or crystalbumin (Furchgott and Ponder, 1940). This substance is adsorbed from a suspension of red cells by glass surfaces, such as the surfaces of the slide and coverslip or the surface of glass beads, and cells freed of it will adsorb it again, quantities of the order of 800 mg. per 100 cc. of cells being involved. If all this were taken up at the red cell surface, it would form a layer only a few molecules thick, but such a layer would have a volume about one-third that of the estimated volume of the red cell membrane as a whole.

2. The spherical form produced by lecithin. A similar disc-sphere transformation, with thorn-apple forms as intermediates, occurs when lecithin is added to red cells suspended in serum, plasma, or saline. The lecithin is most conveniently used in the form of a sol, made by adding 0.5 cc. of a 10 p.c. solution of lecithin in alcohol to 100 cc. of boiling saline. This sol keeps for weeks in the refrigerator, although some of the lipoid may separate out, but the lecithin in it can be estimated from time to time. Again the spheres are formed without any change in volume; they ultimately hemolyse, but not for hours when minimal amounts of lecithin are used. I have recently done a considerable amount of work on the quantitative aspects of this transformation, and the results may be summarized as follows. (1) The quantity of lecithin required to bring about the disc-sphere transformation is remarkably constant for the cells of one species, and for washed human cells the amount needed is about 4 molecules per A^2. of cell surface. This quantity is large, for the smallest cross-section of the lecithin molecule is probably itself about 14 A^2. If the cells are suspended in plasma, about seven-eighths of the lecithin is adsorbed or otherwise combined with the plasma proteins, and only the remaining eighth is available for the cells. (2) The amount of lecithin needed to complete the disc-sphere transformation is about double that required to initiate it; quantities intermediate give forms with crenations of increasing fineness. (3) About half

the amount is needed at 37°C. as at 4°C., so the temperature coefficient is positive, but not large. (3) If the cells are fixed with fixatives such as formol, the amount of lecithin required to produce spheres is increased roughly as the square of the formol concentration. As unfixed cells require a certain amount of lecithin to initiate the change from disc to sphere, the effect of formol is simply to increase this yield-point, a fact which has some bearing on the natural rigidity of the cell and on the increase of this rigidity on gelation.

The disc-sphere transformation can be reversed by washing the cells in untreated plasma, serum, or saline, and the transformation and its reversal can be repeated several times under favorable circumstances, although each repetition involves more and more lysis. The variations in the amount of lecithin required to transform the red cells of different animals has not been fully worked out, but such differences exist.

3. The spherical form produced by rose bengal. Red cells suspended in plasma, serum, or saline undergo a typical disc-sphere transformation without change in volume if small amounts of eosin ($M/10^3$), erythrosine ($M/10^4$), or rose bengal ($M/10^5$) are added in the dark, the effect on washed cells being much greater than on cells in plasma. In the light, smaller quantities of dye are needed, and the quantity of rose bengal which brings about the transformation in the rabbit red cell is such as would cover only about 1/20 of the cell surface, even if it were all concentrated there. The change in shape, in this case at least, is presumably brought about by an effect on a surface component. The spheres can be re-converted into discs by the addition of plasma, and the active component seems to be plasma protein; the re-converted discs show the same stickiness, slight crenation, and mottling as is seen in the case of discs re-converted from spheres between slide and coverglass.

4. Spherical forms produced by other lysins. It is now recognized that all forms of hemolysis are preceded by a disc-sphere transformation, sometimes occurring immediately before lysis, as in the case of saponin, and sometimes a long time before, as in the case of lecithin. What happens seems to be that at a certain stage of the action of the lysin on the cell membrane a "shape component" gives way, and the cell, often quite suddenly, becomes a sphere; after further action a "permeability component" breaks down, and the cell hemolyses. Although this loss of shape and this loss of semi-permeability are two stages of one lytic process, the "shape component" can be destroyed without any important change in the permeability properties. Further, there is no appreciable change in the resistance, capacity per unit area, or frequency dependence of the capacity, when the disc-sphere transformation occurs (Curtis, 1935), nor of the electrical mobility (Furchgott and Ponder, 1940), which appears to be unchanged, indeed, even after the cell is hemolysed in several different ways (Abramson, Furchgott, and Ponder, 1938). These observations lead not only to the conclusion that the "shape component" is distinct from the "permeability component", but that the phenomenon of lysis does not *necessarily* involve the surface of the cell membrane as a whole, and many diverse observations support this view (see Ponder, 1941,a, for a summary of the evidence).

5. The spherical form in hypotonic solutions. When the red cell is placed in a hypotonic solution, it swells, sometimes as much as would be expected if it were a simple osmometer and sometimes less (Ponder, 1940), and in doing so becomes cup-shaped or bell-shaped. If the solution is sufficiently hypotonic it more or less suddenly becomes spherical, and then hemolyses, and it has been shown (Ponder, 1937, Castle and Daland, 1937) that the surface area of this sphere is substantially the same as that of the original disc, although the sphere contains the greater volume (135 - 150 volumes as compared with 100 for the disc). Examination of the cells, now become ghosts, will reveal the fact that they are once more discs, of substantially the same volume as they were initially. The re-assumption of the discoidal form is apparently spontaneous, the forces on the membrane having been relieved by the lysis of the cell. If sufficient salt is now added to make the system isotonic, the discoidal ghosts shrink to about 60 per cent of their volume ("reversal of hemolysis"), showing that they retain some semi-permeability, but after a little while they swell to take up the initial volume once more (Ponder, 1941, m.).

The remarkable fact is that such ghosts cannot be turned into spheres by placing them between slide and coverglass, by adding lecithin, or by treating them with lysins such as rose bengal and saponin. They seem to have undergone a "permanent set" in the shape of discs.

2. The ultrastructure of the cell membrane.

The study of the disc-sphere transformations lead us to the conclusion that the changes are probably due to the action of substances which act at surfaces, and that the component which maintains the shape is probably a surface component. This brings us back to Norris' theory, which demands a kind of "liquid crystal" structure of the cell surface layers. Chemical analysis shows the red cell membrane to be a protein-lipoid complex, and since both X-ray analysis and the methods of polarization optics have shown protein-lipoid com-

plexes in other membranes (e.g., the axon sheath, Schmitt, 1936) to have a micellar organization, it is not surprising to find that the optical properties of the membranes of the ghost also show the presence of an ultrastructure (Schmitt, Bear, and Ponder, 1936, 1938). This structure may be interpreted as consisting of layers of protein oriented tangentially, and layers of lipoid molecules oriented radially, the phosphoric acid group of the cephalin molecules being towards the water side of the interface, and dominating the situation so far as the electrophoretic properties are concerned (Furchgott and Ponder, 1941). Judging from the quantities of protein and of lipoid found in the membrane by chemical means, there may be several such layers alternating with each other (as in the axon sheath), although not in the form of continuous films, for Parpart and Dziemian's (1940) figures show that the amount of extractable lipoid, all of which is contained in the cell membrane as we know it, is not sufficient to make more than a bimolecular layer 30 A. thick. The protein moiety would provide layers with a total thickness of about 90 A., and adding the two contributions together, the thickness of the membrane would work out at about 120 A.

There has been in the past, and still is, considerable doubt as to this total thickness, and this is partly because the analytical figures given by different investigators have not agreed very well with each other, partly because of an insistence on the necessity of continuous molecular films instead of a binding of phospholipoid to protein, which would lead to an orientation of cephalin and lecithin at particular loci around the protein molecules in the membrane (Parpart and Dziemian, 1940), and partly because the contribution of water has not always been taken into account. Estimates of the extent of this contribution range from 10 p.c. of the thickness to 100 p.c. of the thickness; Waugh and Schmitt (1940) give about 25 p.c. Estimates of the total thickness of the membrane of the ghost range from 30 A. (Gorter and Grendel, 1926, Fricke, 1926), to 120 A. (Parpart and Dziemian, 1940), 120 A. exclusive of the contribution of water (Fricke, Parker, and Ponder, 1939), 135 A. at pH 7 and 220 A. at pH 6 (Waugh and Schmitt, 1940), and even higher values. Recently Zwickau (1941) has obtained photographs of fixed and dried membranes of ghosts by means of the electron microscope, and sets the thickness as from 200 to 300 A. If the contribution of water is allowed for, his values would be among the highest yet suggested. The thickness of the membrane in the intact cell may, of course, be greater than it is in the ghost, for substances, perhaps not essential to the resistance, capacity, and semi-permeability of the cell surface may be washed out of it in the process of hemolysis. The anti-sphering substance, which makes up about one-third of the protein content of the membrane of the disc, is one such substance. I therefore feel that it is possible that the cell membrane may approach 500 A. in thickness under certain circumstances, and so reach such dimensions as to be visible, although not resolvable, by the microscope.

3. *The interior.*

The conclusion that the red cell possesses a membrane with an ultrastructure and that the shape is governed by molecular arrangements and forces in the surface layers does not stand in the way of its possessing an interior structure as well, although the views of Rollett and of those who insisted on the complex nature of the surface have often been thought of as mutually exclusive. There are at least four good reasons for thinking that the interior is not occupied simply by a homogeneous solution of hemoglobin and salts.

1. There is a correlation coefficient of only about 0.5 between the density of the red cell and its hemoglobin content. In the absence of hemoglobin, its place is apparently taken by other proteins of about the same density, and these may be the precursors of hemoglobin found in the developing cell. There is quite an extensive literature showing that "proteins other than hemoglobin" are present in the erythrocyte; one of these is the anti-sphering substance, and another is the stromatin of Jorpes, which Boehm believes to be anisodiametric and to fill the cell interior.

2. The classical experiments of "cutting a red cell in half", perforating it with glass spicules, and some of the modern micrurgical observations lead to the conclusion that the cell interior may be gelated under certain circumstances. I have even gone the length of suggesting that such a gelation may contribute to some of the anomalous osmotic properties (Ponder, 1940).

3. When ghosts are placed in solutions of hemoglobin, there is an adsorption of greater quantities of hemoglobin than are likely to be bound at the surface (Ponder, 1941, a). The pigment may be adsorbed on an internal stroma. In this connection, it is well known that it is exceedingly difficult to rid ghosts of the last traces of pigment.

4. The shape of the red cell is not always that of a biconcave disc. In some persons the cells are oval ("ovalocytosis") like the erythrocytes of camels. These ovalocytes show typical disc-sphere transformations (Ponder, 1939). In experimental and other anemias there appear irregularly shaped cells (poikilocytes) which also show disc-sphere transformations, but where the dis-

torted parts of the cell apparently "sphere up" with difficulty, as if there were some restraint. This is very clearly seen in the juvenile red cell, the reticulocyte, in which bands of material, apparently situated in the interior, can be stained with brilliant cresyl blue; these persist unchanged in form after lysis of the reticulocyte, and apparently interfere with the disc-sphere transformation by binding down the parts of the surface to which the bands are attached.

This last observation, that certain parts of the poikilocyte and reticulocyte surface are less mobile than others, brings us back to an important observation by Furchgott (1940, b). He was able, by adjusting the pH of the medium, to make a preparation of cells which would become spheres as alkali diffused from the glass, but revert to discs on the addition of CO_2 (by breathing on them). In such preparations he observed that the biconcavities, and even the larger crenations, appeared when the sphere turned into the disc at the same points at which they were present before the disc turned into the sphere. This provides excellent evidence that the structure of the membrane varies from point to point, and Waugh and Schmitt's leptoscopic observations (1940) lead to the same conclusion.

4. *Conclusion.*

Although it is impossible to set forth all the evidence in a review of this length, I think that a very fair case can be made out for Norris' theory of the shape of the mammalian red cell. It is true that he laid rather too much emphasis on the liquid nature of the erythrocyte, and that he says that "its biconcave shape is due to the operation of physical conditions and not to structural restraint", but it is clear that by this he means internal structural restraint, for he describes the "exquisitely delicate physical pellicle", and it is in this that he supposes the physical conditions to operate. We would speak of an ultrastructure at the surface, and of a special arrangement of molecules and forces between them.

A real difficulty, however, remains, and this is to explain, on some fundamental grounds (and not just by saying "because it was made that way"), why the arrangement of molecules and the forces between them is such as to give rise to the particular biconcave form. This is probably a matter for the physicists and the chemists. There is, however, another line of investigation to which attention is not sufficiently called. The discoidal orthochromatic red cell is derived from the larger reticulocyte, in which there is a network which can be stained with brilliant cresyl blue; this is derived from a nucleated cell, the normoblast, and this again from another nucleated cell, from which hemoglobin is absent, the pro-erythroblast. The discoidal shape seems to be assumed about the time when the nucleus of the normoblast breaks up and disappears, and we ought not to let the accomplishments of physics and spatial chemistry make us forget that the investigation of the shape of cells still lies in the domain of cytology. Remarkably enough, there are few reliable observations, and no reliable measurements, on the shape of the precursors of the erythrocyte, and this is a line of research upon which the biologist can immediately embark with the expectation of adding substantially to our knowledge.

(This article is based upon a lecture delivered at the Marine Biological Laboratory on July 18.)

SOME ASPECTS OF PIGMENT DEPOSITION IN FEATHER GERMS OF CHICK EMBRYOS

Dr. Ray L. Watterson

Assistant in Biology, The Johns Hopkins University

A combined histological and experimental study of the developmental history of pigment cells in the wing skin and feather germs of Barred Plymouth Rock embryos has demonstrated that potential pigment cells, which originate only from the neural crest, begin to migrate into the wing bud epidermis between 79 and 80 hours of incubation. Melanoblasts which have successfully invaded the epidermis in this way, and their derivatives by mitotic division, begin the formation of pigment between 7 and 7½ days, thereby becoming definitive pigment cells; and by 8 days the epidermis of the wing is filled with a network of melanophores. Feather germ formation begins about this time and is characterized by a thickening of the epidermis—first by cell elongation, then by cell proliferation—and by an accompanying condensation of the underlying dermis, until a cap of cells finally protrudes above the surface as a definitive feather germ. Any pigment cells which are present in the epidermis increase in number by cell division and are carried along by the morphogenetic changes in the epidermis. By 10 days the feather germ is considerably larger and is filled with a complex network of pigment cells. These much enlarged melanophores appear to be distributed entirely at random, and, although each is packed with pigment granules, no pigment has been deposited as yet. A few hours later this random distribution disappears, and all pigment

cells in the upper two-thirds of the feather germ become grouped into 10 or 11 longitudinal rows. In order to understand the nature of this redistribution of melanophores, it is necessary to examine the definitive down feather, and to project this structure back upon the feather germ.

Each down feather consists of 10 to 15 barbs held together basally, and each barb bears 2 rows of barbules. Each barb plus its barbules is designated collectively as a barb-vane. If these component parts of the barb-vane are projected back upon the feather germ, they exhibit different spatial relationships from those seen in the definitive feather. Within the feather germ each barb-vane is folded up in such a way that it forms a longitudinal barb-vane ridge projecting inward towards the pulp and bounded peripherally by the feather sheath. The barb lies at the apex, and one row of barbules occupies each lateral margin of each ridge. Since all barb-vanes are folded up in this fashion within the feather sheath, the circumference of the feather germ is divided into as many longitudinal ridges as there will be barb-vanes in the completed feather. It is this breaking up of the walls of the feather germ into longitudinal ridges which brought about the change from the random distribution of pigment cells to a definite distribution into several rows, each row corresponding to one of these ridges. A somewhat earlier stage, characteristic of 11-day feather germs, represents the one time during development that the barbule cells can receive pigment. The barbs have not yet differentiated, and the cell bodies of the pigment cells now lie at the apex of each ridge. Their processes extend peripherally and carry pigment to the barbule cells.

It is the nature of this relationship between the pigment cell and the barbule cell which attracts our attention. Previously the pigment cell has been considered to be almost a micro-injection apparatus capable of injecting pigment granules into passive recipient barbule cells. However, several lines of evidence seem to indicate that a much more active role is played by barbule cells during the pigmentation process.

(1) Pigment cells are loaded with pigment granules at an early stage even before longitudinal ridges have formed. Nevertheless, pigment is not deposited into any epidermal cells until certain of those cells become visibly differentiated in the direction of barbule cells, whereupon they begin to receive pigment.

(2) Before any definite barbule cell differentiation occurs within a ridge, pigment granules accumulate at the tips of pigment cell processes and are pinched off and come to lie freely among the cells of the ridge. Pigment liberated in this manner is later taken up by barbule cells.

(3) Pigment is deposited in each row of barbule cells in a definite sequence. The melanophore process extends past the most centrally located barbule cells and first carries pigment to the most peripheral cells, and then progressively to more axial cells. This is the same order in which barbule cells undergo differentiation. The most peripheral barbule cells are the first to elongate and to form keratin, and only when these visible differentiation processes begin can they receive pigment. As this wave of differentiation spreads progressively toward the pulp, the more axial cells in turn become capable of receiving pigment.

(4) Pigment cell processes appear to be specifically attracted toward barbule cells. Under normal conditions, none are directed toward cells lying between the two rows of barbules. However, occasionally a melanophore process does carry pigment to a group of cells lying in this axial plate region. This at first appears to be contradictory to the idea of a specific attraction, but if the fate of these pigmented cells is followed, they are found to divide into two groups, and then the longitudinal ridge is divided apico-basally between them, whereupon these cells which originated in the center of one barb-vane ridge become distributed between two ridges and are distinguishable as barbule cells in each ridge. Since normally each barb-vane ridge forms one barb-vane, these split ridges must form split barb-vanes, which they do. Thus, the development of these unusual down feathers clearly demonstrates that pigment cell processes are specifically attracted to barbule cells at this time, even when they must leave their usual paths in order to reach them.

(5) Pigment deposition appears to stimulate the melanophores involved to undergo proliferation. If the apico-basal distribution of pigment cells which are undergoing mitotic division is plotted from an 11- and a 12-day feather germ, the mitotic figures are definitely concentrated within a relatively narrow region. It is only within these regions that barbule cells are actively receiving pigment.

(6) Finally, a study of the growth curves of down feathers reveals an interesting relationship. During the first day of its development, the feather germ grows slowly, but beginning suddenly at 10 days and 18 hours of incubation, the germ elongates rapidly, attaining its full growth by 18 days. Strikingly, the onset of pigment deposition coincides exactly with this onset of rapid growth. Lillie and Juhn have estimated that 90% of the axial growth of regenerating feathers is accomplished by cell elongation. It would appear that pigment deposition begins suddenly at that phase of development when barbule cells begin to elongate rapidly.

The down feather of Barred Rocks is solid black in color, whereas the juvenile and adult

feathers of this breed exhibit alternate black and white transverse bars. Willier has demonstrated that rapidly growing juvenile feathers produce a more nearly solid black pattern than more slowly growing ones. The down feather grows more rapidly than any juvenile feather, elongating 5.5 mm. between the twelfth and thirteen days, and it is indeed a tempting thought that this rapid elongation stimulates continuous pigment deposition, so that a solid colored feather results. This hypothesis is strengthened by a recent observation of Mr. James Foulks. If Barred Rock pigment cells from a regenerating feather germ, where they would normally produce a barred pattern, are transplanted into feather germs of White Leghorn embryos, they deposit a solid black pattern in the host down feathers.

These several lines of evidence may indicate that barbule cells are more important in the pigmentation process than we have previously realized.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 22.)

THE INFLUENCE OF HORMONES ON THE DIFFERENTIATION OF MELANOPHORES IN BIRDS

Dr. Howard L. Hamilton

Department of Biology, The Johns Hopkins University

An examination of regenerating feathers from birds having red and black in their plumage (e.g., New Hampshire Red fowl, Robin) shows that both red and black melanophores are responsible for the pigmentation. However, when explants of skin from embryos of these birds are grown in a tissue culture medium consisting of embryonic extract and blood plasma, then black melanophores appear, but red ones occur very infrequently in the tissue. If sex hormones are added to the culture medium, then many red melanophores as well as black ones differentiate in the explant. These effects on melanophores are similar to those which are obtained when hormones are either added or removed (by castration) from birds (see Domm, '39, for a discussion of plumage color changes).

Several criteria indicate that red and black melanophores are two discrete types of cells: (1) the pigments are of different colors—orange-brown and black, (2) the granules are subspherical or pebble-shaped in red melanophores and rod-shaped in blacks, (3) red melanin granules partially dissolve so that their boundaries become blurred when fixed in solutions containing picric and acetic acids, whereas black melanin is insoluble in such reagents, (4) the cytoplasm of red melanophores is very fluid as compared with black melanophores, because red granules show extreme Brownian movement, while black ones move slowly due to protoplasmic streaming, and (5) the two types of pigment cells react differently to the various hormones.

In general, the sex hormones (estradiol dipropionate, estradiol monobenzoate, testosterone propionate, estrone) increase the number of red melanophores which differentiate in treated explants from the New Hampshire Red and Rhode Island Red breeds. Sesame and olive oils also produce an appreciable stimulation, possibly due to traces of sterols. The responses of black melanophores from the red breeds to these same hormones are more involved. The two esters of estradiol inhibit pigment cells, but estrone and testosterone favor their differentiation.

Because of the similarity of its chemical structure to that of testosterone, the adrenal cortical hormone, desoxycorticosterone acetate, was used on explants from red breeds. The result was a nearly complete inhibition of both red and black melanophores. A similar reduction in the number of melanophores occurred when skin from White Leghorn and Barred Plymouth Rock fowl was grown in the presence of the cortical hormone. A more extensive study on the Barred Rock, which possesses only the black type of melanophore, showed that sex hormones as well as the cortical hormone decrease the number of pigment cells. The extent of the inhibition depended somewhat on the age of the skin when isolated. Young tissue (5-6 days) often yielded no melanophores when grown in the presence of hormone, but more usually (when the hormones were dissolved in sesame oil) there were expanded melanophores, but fewer of them than in controls. Skin from older embryos (7-8 days) already contains differentiated melanophores and localized centers where feather germs are to form. However, when explants are treated with hormones, most of the differentiated melanophores clump and degenerate, and only the newly-appearing ones persist as expanded cells. Furthermore, melanophores aggregate at loci where feather germs should arise, but no structures are formed. This result cannot be explained on the basis of a general growth inhibition of all cells, because the zone of outgrowth from the explant is approximately the same size in both the treated and control cultures. When crystalline hormones are used there is a reduction in number of melanophores, but this is not as striking as the delay in their differentiation. The red pigment cells in the treated portion of the iso-

(*Continued on page* 112)

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris I. Gorokhoff and Judy Woodring.

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CONFERENCE AT THE UNIVERSITY OF CHICAGO

As part of the program celebrating the Semi-Centennial of the founding of the University of Chicago, a Conference on the Training of Biologists will be held. According to an announcement of this conference, of which Dr. Paul Weiss is chairman, the preparation of a prospective scientist for his future task, to promote, propagate and apply scientific knowledge, requires careful planning based on insight into the nature of science and its aims, methods, potentialities and limitations. To discuss the fundamental problems arising in the planning of the education of students in the Life Sciences, twenty-four scientists and educators, representing a variety of disciplines bearing on these problems, will gather for a free exchange of views and integration of ideas.

The conference will be held in five sessions, round-table fashion, on Thursday, September 18, Friday, September 19, and Saturday morning, September 20. The general topics are as follows:

First Session: Introduction—Presentation of educational programs in biology currently in operation in some major universities. *Second Session*: Contribution to the training of biologists from the physical sciences and other related disciplines. *Third Session*: The basic educational needs of the biologist. *Fourth and Fifth Sessions*: The specific preparation of biologists for professional specialization (research, teaching, medicine, etc.)

The morning sessions will start at 9:30 A. M., the afternoon sessions at 2:00 P. M.

LIST OF PARTICIPANTS

From the University of Chicago:

Paul A. Weiss, Chairman, Department of Zoology; Emmet B. Bay, Department of Medicine; John M. Beal, Department of Botany; William Bloom, Department of Anatomy; Anton J. Carlson, Department of Physiology; Merle Coulter, Department of Botany; Earl A. Evans, Jr., Department of Biochemistry; Ralph W. Gerard, Department of Physiology; Victor Johnson, Dean of Students, Division of Biological Sciences; Wilton M. Krogman, Department of Anthropology; George K. K. Link, Department of Botany; Carl R. Moore, Department of Zoology; William H. Taliaferro, Dean, Division of Biological Sciences; Ralph W. Tyler, Department of Education.

From Other Institutions:

Detlev W. Bronk, Professor of Biophysics and Director of the Eldridge Reeves Johnson Research Foundation, University of Pennsylvania; Karl S. Lashley, Research Professor of Neuropsychology, Harvard University; Dwight E. Minnich, Professor and Chairman of the Department of Zoology, University of Minnesota; Karl P. Schmidt, Chief Curator of Zoology, Field Museum of Natural History; Francis O. Schmitt, Professor of Biology and Head of the Department of Biological Engineering, Massachusetts Institute of Technology; Edmund W. Sinnott, Sterling Professor of Botany, Yale University; Laurence H. Snyder, Professor of Zoology, Ohio State University; C. V. Taylor, Herzstein Professor of Biology and Dean of the School of Biological Sciences, Stanford University; Benjamin H. Willier, Henry Walters Professor of Zoology and Chairman of the Department of Biology, Johns Hopkins University.

DATES OF LEAVING OF INVESTIGATORS

Baker, Gladys	July 21
Illick, J. T.	July 23
Kreezer, G.	July 25
Miller, J.	June 30
Ronkin, R. R.	July 25
Rothstein, A.	July 14
Stowell, R. E.	July 27
Warner, E. N.	July 25
Weaver, Margaret A.	July 26

ADDITIONAL INVESTIGATORS

Benedict, Dora Milton Acad. (Mass.). Br 309.
Birmingham, L. grad. asst. biol. Rochester. OM 39. Dr 3.
Boyd, M. J. asst. prof. biochem. Cincinnati. Br 341.
Castelnuovo, Gina zool. Missouri. lib.
Cole, R. M. grad. fel. biol. Harvard. OM 39. Ka 3.
Claude, A. assoc. Rockefeller Inst. Br 206.
Davson, H. assoc. prof. phys. Dalhousie. Br 107.
Gray, I. E. assoc. prof. zool. Duke. lib.
Hendricks, Anne L. Cincinnati Med. Br 341. A 202.
Hopkins, Marjorie G. grad. asst. zool. Mt. Holyoke. OM 39. H 3.
Humm, Frances D. grad. fel. emb. Yale. lib. D 311.
Keosian, J. asst. prof. biol. Newark. Br 315.
Morgan, Lilian V. (Pasadena, Calif.). Br 320.
Muir, R. M. grad. fel. bot. Michigan. Bot. Dr 6.
Saunders, Grace grad. fel. biol. New York. OM 39. H 9.
Shapiro, H. S. techn. biol. Williams. OM 26. Dr 15.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
August 2	11:57	.....
August 3	12:41	12:57
August 4	1:42	1:57
August 5	2:39	2:53
August 6	3:32	3:46
August 7	4:20	4:35
August 8	5:08	5:23
August 9	5:51	6:09

In each case the current changes approximately six hours later and runs from the Sound to the Bay.

ITEMS OF INTEREST

DR. A. J. WATERMAN, who is instructing in the invertebrate course, has been promoted from assistant to associate professor of biology at Williams College.

DR. HENRY EMERSON, instructor in anatomy at the University of Michigan, has been appointed instructor in biology at Amherst College.

DR. MARY SEARS, who is at present working in the Woods Hole Oceanographic Institution, has received a Faculty Fellowship at Wellesley, and in addition has a grant from the Committee for Inter-American Artistic and Cultural Relations to conduct research work on Chincha Island, Peru. The work will consist of an investigation of plankton in connection with the feeding habits of Guano birds. Beginning August 15 her study will continue about eight months, after which time she will return to the Oceanographic Institution.

DR. FRANK A. BROWN, JR., associate professor of zoology at Northwestern University, is teaching a course in comparative physiology and another in invertebrate hormonal mechanisms at the University of Chicago.

DR. SAMUEL A. MATHEWS, who instructed last summer in the invertebrate zoology course, spent the month of July at the Scripps Institution of Oceanography.

DR. RALPH H. CHENEY, who has worked at the laboratory in previous summers, is on the Pacific coast visiting the marine biological stations at La Jolla, Corona del Mar, Pacific Grove, and Friday Harbor.

DR. JAMES A. MILLER, who in previous years conducted work at the Marine Biological Laboratory, is on the staff of the invertebrate course at the Mount Desert Island Biological Laboratory this summer. In addition to lectures on the coelenterates, flatworms, and annelids, Dr. Miller is making a Kodachrome moving picture of the activities of the course. Before leaving the East for the University of Michigan, he expects to return to Woods Hole for several days.

MR. J. PAULL, who was registered for the invertebrate course did not come to Woods Hole; his place has been filled by Miss Louise E. Gross of Smith College.

A new marine biological laboratory is being planned by the University of Texas. Located on the Texas coast of the Gulf of Mexico, the Laboratory has been granted $25,000 by the General Education Board. Additional funds will be necessary, however, before construction can be started.

The *Atlantis* returned from dry dock at the end of this week, and will leave Sunday or Monday for the other side of the Gulf Stream.

DR. AND MRS. CHARLES O. WARREN visited Woods Hole over the weekend. Both Dr. Warren and his wife, the former Katherine S. Brehme, have worked at Woods Hole in the past. They are spending the summer at the Biological Laboratory at Cold Spring Harbor.

MISS ANNABELLE BROOMALL was married to Dr. Richard Horn on June 11 in Wilkinsburg, Pennsylvania. She is working at the Laboratory on the genetics of a parasitic wasp, and this fall will work for her doctor's degree at the University of Pittsburgh. Her husband is now interning in the Pittsburgh Medical Center.

At the weekly staff meeting at the Woods Hole Oceanographic Institution on Thursday evening, Dr. Theodor van Brand, of the Catholic University of America, spoke on "Experimental Studies upon the Nitrogen Cycle in the Sea."

Motion pictures on "Seals in Alaska" were shown Thursday evening in the M.B.L. Auditorium. The film was provided by the Fish and Wildlife Service and explanatory comments were made by Dr. P. S. Galtsoff.

Mr. Lower's Movies of Aquarium Life and Children this summer will be shown on Friday, August 8, 3:00 P. M. in the Schoolhouse for the benefit of the microscope fund. 20c children, 40c adults. The Annual Exhibition of Children's Work will take place from 2 to 5 P. M., Friday, August 8.

The program of the phonograph record concert at the M.B.L. Club Monday night is as follows: Beethoven, "Egmont Overture;" Prokoffiev, "Peter and the Wolf;" intermission; Tschaikowsky, "Symphony No. 4."

M.B.L. TENNIS CLUB

The officers of the Tennis Club will appoint a committee to run a tournament of events in ladies' and men's singles and doubles, provided enough players care to enter. The entries must be in by Monday, August 4, and drawings will be made that evening. Play will begin immediately in order to finish the finals by the middle of August. Entries should be made on the sheets posted by the Mess court.

The courts are less crowded than usual this summer, and the club needs the support of the community to reduce its indebtedness. The havoc caused to the new beach courts by the tidal wave in 1938 brought about heavy expenditures for reconstruction. During the past two seasons several club members have saved the club about two hundred dollars by putting the surface into playing condition. In addition, the club is grateful to several people who have given generous aid and so helped to reduce the club debt. —*D. E. L.*

STUDIES ON THE LIFE HISTORY OF *SIPHODERA VINALEDWARDSII*, A TREMATODE PARASITE OF THE TOADFISH

Drs. R. M. Cable and A. V. Hunninen
Purdue University and Oklahoma City University

Experimental studies on the life history of *Siphodera vinaledwardsii* (Linton) have demonstrated that this trematode is related to the Heterophyidae as postulated by Manter, Price, and Wilhelmi on the basis of morphological and serological investigations. The definitive host in the Woods Hole region is the toadfish, *Opsanus tau*, practically all of which are infected. The small marine snail, *Bittium alternatum*, serves as the molluscan host in which the cercariae develop in simple, elongate rediae. The cercaria is a pleurolophocercous form of an unusual type since the tail is inserted ventrally and coiled when at rest, the fourteen penetration glands have two instead of the usual four bundles of ducts in the region of the oral sucker, and the excretory formula is $2[(2+2)+(2+2)] = 16$ flame cells. The cercariae penetrate and encyst in various species of flounders, developing into infective metacercariae in approximately two weeks. Metacercariae occur in the fins, body wall and even the myocardium of the fish. Feeding experiments thus far completed indicate that toadfish become infected by eating fish containing metacercariae. Three toadfish, isolated for four weeks, were fed fish containing 13-day metacercariae. Two of these have been examined and found to contain large numbers of very young worms in addition to a few mature specimens from previous natural infection.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 15.)

THE INFLUENCE OF HORMONES ON THE DIFFERENTIATION OF MELANOPHORES IN BIRDS

(Continued from page 109)

late contain fewer melanin granules, so that they appear lighter-colored, and granules are not eliminated as soon from them. Though chronologically of the same age, these treated melanophores are physiologically younger than the controls.

In summary, it is seen that genetic differences in the precursor cells must determine whether they become red or black melanophores, since both kinds develop adjacent to one another in the same piece of tissue and in the same clot. However, certain environmental factors must influence which of the two kinds of melanophores will predominate. For example, fast-growing feathers are mostly black in the Rhode Island Red breed, so that physiological differences in feather germs must favor the differentiation of one or the other kind of pigment cell. This study also shows that hormones (and probably other sterols) increase or decrease the numbers of each kind of melanophore. Hormones apparently act directly on melanophores, since they are fairly well-isolated *in vitro* from other cell types which might affect them. The evidence suggests that the hormone exerts some intracellular metabolic effect so as either to catalyze or inhibit melanin synthesis. Apparently there are no transitional stages between red and black melanophores; hormones do not cause one kind of pigment cell to change into another. Red melanophores are not produced by stopping normal melanin synthesis at a red stage. If this were true, all black melanophores should pass through a red phase in their formation, and this is not observed. Hormones cause the appearance of red melanophores only in those birds which have genes for red pigment (i.e., possess red-type precursor cells). These precursors or melanoblasts remain latent in the tissue until conditions are favorable, either in cultures or in feather germs, for them to synthesize melanin.

An examination of human hair follicles shows that both red and black melanophores are present in some individuals, and that different proportions of these two kinds of pigment cells account for the varying shades of brown, sandy, and red hair. Dark brown and black hairs contain only black melanophores. The similarity of these two kinds of mammalian pigment cells to those of birds suggests that they, too, might be influenced by hormones. Graying in hair and whiteness in feathers are similar in that both are caused by loss of pigment cells (due to decreased viability of melanophores or adverse environmental conditions in the follicle) so that those few which remain are unable to produce enough pigment to color the hair or feather.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 22.)

INVERTEBRATE CLASS NOTES

We invertebrates, fifty-five in all, representing forty-eight colleges and universities, had our first meeting Thursday evening, July 24. At this time, jovial Dr. Bissonnette introduced our instructors, explaining the workings of the course, warned us about the tides, poison ivy, and making sea water do the work of formalin.

Protozoans were the subject of three lectures by Dr. Waterman, in which he not only reviewed the structural and ecological aspects, but also some of the important research problems of this group. For us beginners at least, the lab work became more difficult as we turned from attached forms to parasites and free-swimming forms. The lab was by no means deserted over Sunday, some of us striving to cover a respectable minimum, others to take advantage of the abundant material. Indeed, we have become quite familiar with the customary peroration: "There is sufficient material for a week's work. Please hand in your lab records by tomorrow noon."

Dr. Lucas had the tough assignment of crowding into a single afternoon a comprehensive lecture on the somewhat anomalous Porifera, to be followed by our investigations of the several types available. To carry out our end of the job, many of us worked well into the night. The failure of the power supply at one A. M. sent the last investigators groping to bed.

Spicules and choanocytes duly observed and recorded, we have turned our attention to polyps and medusae. Guided by Dr. Crowell's well organized lectures we have been examining the diverse types and phases of hydroid life.

Our first two days were further enlivened by field trips, one to Stoney Beach, the other to Lackey's Bay. Equipped with the "Ark"—fully as capable as its Biblical prototype at taking, two by two or as fast as we find them, any and all marine invertebrates—watch glasses, forceps, hand lenses, we readily found representatives of most of the invertebrate phyla. Identification was another matter, one largely in the hands of our omniscient instructors. By the second trip however, the "recording angel" was hard put to keep track of the staccato calls of "Amaroucium", "Nassa trevetata," "Diapatra," scarcely audible above the other sounds associated with a successful field trip.

As we're a social noisy group, we received the news of the M.B.L. Mixer with enthusiasm. Few of us went with the sole purpose of meeting celebrated investigators, but the rest of us preferred the frivolous chatter and dancing. With our background in Protozoology we added an appropriate (purely scientific, of course) finish to the day by swimming in the biluminous sea. Several active souls finished the evening in the lab, boiling unwanted lobster claws and playing bridge.

During lab hours (i.e., any time) we hear the results of John Osmun's newly (quite newly) formed quartet with Bob Porter, Howy Miner, and Sid Pond. The one person that will effectively interrupt them is Gary Metcalf of the collecting crew who never fails to praise the crew's soft ball team. We have met the challenge by organizing a volunteer eleven (not to mention a complete girl's team). Dr. Rankin has promised to be our first base man. This stroke of luck should remove all doubt concerning the final scores. A merciful rain postponed our first game.

—*Louise Gross and Bill Batchelor*

EFFECT OF SEA WATER ON THE RADIOSENSITIVITY OF *ARBACIA* SPERM

(Continued from page 101)

TABLE I.

Percentage Fertilization

Amount of radiation: 76,500 roentgens.	Irradiated				Controls			
Conc. of sperm:	"Dry"	1:5	1:10	1:100	"Dry"	1:5	1:10	1:100
Time of insemination after irradiation:								
60 min.:	100	100	87	3	100	100	100	100
90 min.:	98	62	19	2	99	100	91	98

from the above data that another indication of increased sensitivity at greater dilutions is a decrease in the ability to survive over a period of time. It appears that some sperm, not killed instantaneously, may be injured so that they die later.

Because of the possibility that the increase in sensitivity might be due to production of toxic materials in the water, irradiated sea water was added to sperm but no definite injury was observed.

A quantitative study of the change in resistance

Table II.

Amount of radiation:	None	1,160 r	2,120 r	3,180 r	4,240 r	5,300 r
Percent. fert.:	100	69.5	45.0	35.4	41.5	33.8
Conc. Sperm 1:2000 Percent. fert.:	100	7.0	7.1	3.5	13.2	7.95
Amount of radiation:	None	1,195 r	2,390 r	4,780 r	9,560 r	19,120 r
Conc. of Sperm 1:10 Percent. fert.:	100	100	100	100	93.0	93.0
Conc. sperm 1:1000 Percent. fert.:	100	73.0	63.8	36.6	7.1	1.77
Amount of radiation:	None	1,190 r	2,380 r	4,760 r	9,520 r	19,040 r
Conc. of Sperm 1:100 Percent. fert.:	100	97.2	98.3	99.3	92.1	56.9
Conc. of Sperm 1:1000 Percent. fert.:	100	94.7	94.4	21.7	6.7	0
Amount of radiation:	None	5,600 r	11,200 r	22,400 r	44,800 r	89,600 r
Conc. Sperm 1:20 Percent. fert.:	100	86.0	86.0	68.5	48.8	40.7
Conc. Sperm 1:200 Perct. fert.:	100	12.6	1.5	0	0	0

of the sperm at various concentrations was made in the following manner. Sperm were collected in as concentrated a form as possible, such sperm being considered as "dry" or 100% sperm. The desired dilutions were made from the same lot of "dry" sperm, and, after 15 minutes, were irradiated. Following irradiation, fertilization tests were made. Each lot of sperm was diluted to the same concentration before insemination in order that the same amount of sperm would be used in each case for the same number of eggs. The amount of sperm added to the eggs was not sufficient to give 100% fertilization. In this manner, an excess of sperm was avoided and small changes in ability to fertilize could be detected. At least two lots of eggs were fertilized in each case and several counts of each were made. The final counts were made at the time when most of the fertilized eggs were in the two cell stage. The results were expressed as percentage fertilization (as compared to controls). As shown in Table II, the ability to fertilize is markedly altered by the proportion of water present during the irradiation. It should be noted that the dilution experiments were performed in pairs in order to prevent possible differences due to variation in radiosensitivity from one lot of sperm to another. Therefore the values to be compared are those obtained by different dilutions in the same experiment and not necessarily from one experiment to another. However, the results of all of these experiments are in general agreement.

Some experiments have been performed in an effort to obtain some information regarding the mechanisms controlling the change in radiosensitivity. It has been found that dilute sperm suspensions irradiated immediately after the addition of water (when the rate of oxygen consumption is high) are more susceptible than sperm that have been in sea water for 30 minutes (at which time the rate of oxygen consumption has dropped to a lower level). This, together with the fact that the addition of sea water greatly increases the activity and rate of oxygen consumption, indicates that these factors play a part in determining the radiosensitivity of the sperm. Other factors are being investigated in an attempt to elucidate the mode of action of radiation on the fertility of sperm.

Preliminary experiments on *Nereis* sperm indicate a similar relation between dilution with sea water and radiosensitivity.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 29.)

THE COLLECTING NET

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EFFECT OF AZIDE ON *CYPRIDINA* LUCIFERIN

DR. AURIN M. CHASE

Physiological Laboratory, Princeton University

Luciferin is the substrate in the bioluminescent reaction. In the presence of an enzyme, luciferase, and of oxygen in solution, light is produced. Reversible oxidation of luciferin can occur in the absence of luciferase but there is no luminescence in this case. (E. N. Harvey, *Ann. Rev. Biochem.*, 1941.)

The luciferin and luciferase used in the present experiments were obtained from the crustacean, *Cypridina hilgendorfii*. In the thoroughly dried organisms the luminescent materials remain stable practically indefinitely. The luciferin was extracted and purified by the method of Anderson (*J. Gen. Physiol.*, 1935) and the luciferase was partially purified by prolonged dialysis against distilled water. The reaction mixtures, of pH 5.4 and 6.6, were made up from phosphate buffer, 0.2 M. and the sodium azide solutions were also made up in these same buffers. Luminescence was (*Continued on page* 131)

M. B. L. Calendar

TUESDAY, August 12, 8:00 P. M.

Seminar: Dr. W. Trager: "Studies on Conditions Affecting the Survival in vitro of a Malarial Parasite."

Dr. Charles Hassett: "The Effect of Dyes on the Response to Light in Peranema."

Dr. J. O. Hutchens: "Utilization of Ammonia by Chilomonas paramecium."

Miss Virginia Dewey and Dr. G. W. Kidder: "The Possibility of Thiamine Synthesis by Ciliates."

FRIDAY, August 15, 8:00 P. M.

Lecture: Dr. Franz Schrader: "The Mitotic Movements of Chromosomes."

PROTEINS IN ACTION

DR. DOROTHY WRINCH

Professor at Amherst, Smith and Mount Holyoke Colleges

One of the most interesting characteristics of this golden age of biological research is the way in which a new synthesis of ideas is emerging, as the result of the immense progress which has been achieved by specialist investigators working in a score of highly technical biological fields and the rise of the new science of structure chemistry. The original barriers between morphological and functional studies are giving way before the realization of the fact that function can only be understood in terms of structure, so that structure, far from being the concern only of certain specialists, is seen to be of the first importance in a great variety of attacks on biological problems. Biological systems indeed are now seen to be superlatively fine examples of functional architecture. Simultaneously structure chemistry, the science which studies the geometry of intra- and inter-molecular atomic arrangements, has come of age. This

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ALONG WATER STREET DURING THE 1938 HURRICANE

Above: The M.B.L. Club House at the height of the storm, with the Woods Hole Oceanographic Institution behind it. The building between the two telephone poles at the left is Rowe's Drug Store, while that to the right of the Oceanographic building is the Institution's pump house.

Below: A churning mass of wreckage being tossed over the sea wall, photographed from the steps of the Yalden Sundail. At the extreme right is the M.B.L. pump house. On the horizon may be distinguished Lawyer Hathaway's yacht "Rosemay," which battled the storm successfully by running its engines at full speed throughout the hurricane. (Photographs by L. A. Baker)

new science, with which the names of Langmuir, Lewis, and Pauling in this country, and W. H. Bragg and W. L. Bragg in England will always be associated, has already accomplished for many inorganic materials, such as diamond, graphite, the silicates, and water, among countless others, just the type of analysis which is required for the deeper understanding of biological materials. It has shown how mechanical and physical properties can be explained in terms of atomic arrangements. The hardness of diamond and the flakiness of graphite, the softness of naphthalene, the water-holding capacity of the clay minerals, the variation in properties in the different classes of silicates, for example the platey character of the micas and the fibrous nature of the amphiboles—all can be and indeed have been seen to be direct consequences of atomic patterns. The new synthesis in biology now developing has as part of its objective the strictly similar task of the analysis and interpretation of biological data in atomic and molecular terms. In innumerable ways the triumphant transition from millimeters to Ångströms of the structure chemist working with inorganic materials and the simpler organic substances offers pointers to the biologist so that he may in due time accomplish a similar transition, the narrower transition from the micron, even perhaps (with the help of the electron microscope) the decimicron, to the Ångström world.

Tonight I shall ask you to review with me recent findings in a number of experimental fields which can be interpreted in terms of structure chemistry and to consider a number of specific suggestions regarding the possible nature of biological structure systems which result.

Cytoplasm in Focus

Structure problems in biology find a focus in the nature of cytoplasm in general including particularly the biologically functioning membranes, the cell plasma membranes around all animal and plant cells, the nuclear membrane and the membrane of red cells. A great deal is already known as to the mechanical and physical properties of these systems, something also of their chemical nature. Perhaps the most striking data of all are those which indicate an inherent dichotomy in the nature of cytoplasm in general. Thus if, because of its high water content, we wish to consider cytoplasm as a liquid system, we must admit, as Frey-Wyssling and others have pointed out, it is a very anomalous one, since it does not satisfy the usual criteria. It is not inelastic, so it is a non-Newtonian liquid. It does not have a constant viscosity at a given temperature, so it is a non-Poiseuillian liquid. Small particles in cytoplasm do not rise or fall with constant velocity, so it is a non-Stokesian liquid. It is not under all circumstances optically isotropic, so in a fourth respect it forfeits its title to being considered an ideal liquid. Furthermore, cytoplasm has a certain rigidity; it evinces under appropriate circumstances a certain constancy of shape; it possesses some tensile strength.

Any attempt to think of cytoplasm in terms of ordinary ideas of solid structures is equally unsuccessful. Cytoplasm is extremely deformable and very flexible. It is characterized also by the power to shrink and swell reversibly on the grand scale, its essential structure, upon which its functional behavior depends, apparently remaining undisturbed. It has a huge water content which approaches even 97 per cent in the case of certain cells and the water easily allows the passage of certain substances, though not of others.

This fact draws attention to the most characteristic property of all, namely the extreme specificity of the cytoplasm as evinced by its permeability properties in general and in other ways, a fact which indicates the necessity for a totally different type of structure from that of classical chemical systems, whether thought of as solids or liquids. The central points about cytoplasm were put in the clearest manner by Wilson many years ago. "Nothing is more remarkable," said Wilson writing several decades ago, "than that a thing so delicate and plastic should run so true to form through countless generations. How this is possible we can hardly imagine. We can but record the observed fact that it is effected by an inherent power of adjustment and self-regulation which holds the cell fast to its own type." What then is the nature of this structure by means of which, as Wilson says, "within certain well-defined limits the activities of every cell are of specific type," such that "the muscle cell, the nerve cell or the gland cell displays its own characteristic performance"?

Proteins, the Clue

The clue to the mystery is not far to seek. It resides in this fact: no consideration of any structure problem in biology or medicine can be complete unless due account is taken of the protein constituent. I wish specially to make clear that no one with any appreciation of the recent advances in knowledge of the parts played by many

The Collecting Net was entered as second-class matter July 11, 1935, at the Post Office at Woods Hole, Mass., under the Act of March 3, 1879, and was re-entered on July 23, 1938. It is devoted to the scientific work at marine biological laboratories. It is published weekly for ten weeks between July 1 and September 15 from Woods Hole, and is printed at The Darwin Press, New Bedford, Mass. Its editorial offices are situated in Woods Hole, Mass. Single copies, 30c by mail; subscription, $2.00.

non-protein components in living systems could be willing to assert that the protein alone is the whole story. We have only to think of the importance of the lipoid component in red cell membranes and of the carbohydrates, as for example in the pneumococcal polysaccharides, to see the necessity for this qualification. This aspect however has not lacked emphasis. Since the turn of the century there has been a tendency to ignore proteins in physiological problems because exact studies on simpler substances of biological importance have naturally taken precedence. In consequence there has been, in some quarters at least, a tardiness to assess at its real value the role of proteins in biological structure problems.

Thus enzymes were denied protein status as late as 1926 by no less an authority than Willstätter. Curiously enough, in this same year urease was crystallized by Sumner at Ithaca and insulin was crystallized by Abel at Hopkins. Proof that many enzymes are proteins has since been given by Northrop and others. Certain cellular respiratory enzymes which operate through a prosthetic group depend for their specificity on the protein portion of the molecule. Further, several vitamins have been shown to be prosthetic groups which associate with proteins.

Similarly, it has recently appeared that most hormones either are proteins, e.g., insulin, the parathyroid and pituitary hormones, or else exist in the body in combination with a protein, e.g., thyroglobulin. The sterol hormones are an important group for which evidence of protein combination is lacking, but it is interesting to note recent evidence for a combination between cholesterol and pneumococcal hemolysin.

The startling demonstration that the viruses are complex nucleo-proteins has also been a development of the last few years. The remarkable property of the viruses of being able to enter cells and increase in concentration, presumably by an autocatalytic process, puts these molecules on the border line between the living and the inanimate. It may be permissible (as Muller, for example, has maintained) to picture the viruses as the prototype of the genic material itself, which is able to go further and elaborate a cellular environment. These developments mean that the time is ripe for a new appraisal of the significance of proteins in biology and medicine. Proteins can no longer be regarded as relatively inert materials "which exert a colloid-osmotic pressure"; they are emerging as the key substances in physiological processes. They are the key even in the sense that many other biologically-active molecules may owe their significance to their relationship to the proteins. It is my opinion that the realization of all the implications of these facts will mark the beginning of a new era in the biological sciences.

Structure Forming Proteins

But the question then arises as to how proteins play this trancendent role in biological systems. The answer has been stated with admirable precision by R. S. Lillie over twenty years ago when he surmised that "the specific characteristics of every animal or plant may be determined ultimately by their structure-forming proteins." We cannot doubt that proteins are the structural units which, with some other subsidiary materials, build the structures, which the histologists measure in microns, out of the ordered atomic patterns, which chemists measure in Ångströms. There can be little doubt that proteins, in their structure-forming capacity, are the main components of the conductile and contractile mechanisms, and of the cell machinery involved in the process of secretion and absorption. An understanding of the processes involved in normal growth and the healing of wounds naturally requires a knowledge of the mode of synthesis of tissue constituents, and therefore also of protein synthesis.

The recent developments in our knowledge of enzymes, hormones, viruses and genes to which I have already referred are indeed a striking fulfillment of the judgment of a philosopher of the last century who said, already in 1878: "Life is the mode of existence of protein substances." Just how minutely, recent accessions of knowledge of what we may call "the biology of the proteins" allow us to work out the picture of the proteins as the essential "structure-forming" components of living systems, I shall now try to show, even if only in the barest outline.

The main sources of our information are the physical chemistry of proteins, studies of antibodies and the crystallography of native proteins. While these are by no means the only sources of information relevant to our investigation of the "structure-forming proteins," I would emphasize one important aspect. All these techniques have proved themselves capable of yielding information about *native* proteins. In the attempt to understand the nature of such biologically active systems as cytoplasm and plasma membranes, immense emphasis in recent years has been laid on the surmises and suggestions contained in early work on dead proteins such as wool, silk and gelatin, some of which have recently been retracted by the leading worker in the field, W. T. Astbury. Even if the whole subject of the structure of these inert proteins were not in a complete state of flux, there is no reason to think that this is a fruitful source of inspiration for those concerned with living systems, since these dead proteins have none of the properties which physiologists and biologists require of actively functioning proteins. It seems to be little realized how far developed is the present day picture of the native protein, and

how fundamentally it differs from what is known about the grosser properties of dead proteins and from what has been suggested, admittedly on the basis of very inadequate X-ray and chemical data, for the atomic structure. I understand from the Director that it is not necessary that I try to hide from you the fact that, in my opinion, there is a very real crisis in this field of protein structure today. The tradition regarding these Evening Lectures was in fact laid down in 1893. "Somewhat greater freedom in the expression of opinion than might be expected in strictly scientific communications must be permitted," writes the first Director of the Laboratory in the preface of the first of the familiar blue volumes of "Biological Lectures." "In fact it is one of the leading objects of these Evening Lectures to bring forward the unsettled problems of the day and to discuss them freely." Discussing, then, this crisis freely, I would offer it, as my opinion, that, of all the elements in the complex protein field today which are holding up the progress of the understanding of structure problems relating to living matter, the one most responsible for the present deadlock is the hypothesis that polypeptide chains are the essential constituents of native proteins. The recent developments I mentioned make it quite possible that the picture of the structure of hair and the rest may need the most thoroughgoing revision, far beyond the modifications recently suggested in *Nature*. These modifications replace one naïve picture based upon insufficient evidence by another, hardly less naïve, and equally ill-grounded in fact. Be the situation in this field as it may, there seems to me to be no question but that the polypeptide chain hypothesis of hundreds of residues handcuffed in pairs is not only unproven but extremely mischievous, mischievous in its inhibiting effect upon attempts at protein synthesis now long overdue, mischievous also in the influence on the design of experiments in more than one field of physiology. Pressed to define where they stand on the polypeptide chain theory, there prove to be few protagonists of this simple picture who will not admit that the chains (if present) must be interlinked in definite patterns. Yet the fact that this admission leads directly to two or three dimensional patterns of the characteristic residues and all the far reaching implications of this obvious idea remain almost universally unappreciated.

Tonight, drawing the most definite of lines between dead and dying proteins and biologically-active native proteins, I propose to assume that the proteins in cytoplasmic and membrane structures are in the latter form. It may not be possible, in the present crisis, to make out a watertight case for the assumption, but it seems difficult indeed to see where, in living organisms, they would be in the native state if not here. It appears, however, legitimate for the unprejudiced observer to read a striking confirmation of the assumption into the recent work of Ballentine and Parpart who found that erythrocytes subjected to crystalline trypsin at pH 6.8 showed no change in permeability even after 24 hours exposure to the enzyme, a result which incidentally surely should cause acute embarrassment to polypeptide chain supporters.

The Plain Unvarnished Facts

If then we wish to understand the present day picture of the native protein, let us first consider the results obtained by Svedberg with his ultra centrifuge. This work opened a new era in the understanding of proteins, for contrary to the expectations of many whose techniques consisted in first tearing to pieces the delicate structures of these biologically-active substances and then studying the resulting chaos, he found that native proteins are made up of well-defined molecular species. Of course the air-tight proof of molecular status for any one protein involves investigations of the highest delicacy, including above all solubility studies. But by and large the picture of protein units as genuine megamolecules emerges unmistakably from his researches. There also emerges a second characteristic of proteins, namely the dependence of their stability upon interlinking or association with other molecules or ions. The other molecules always, so far as we know, include water; many other types of molecules, including other proteins, also play a part. This seems to be the meaning of the phenomenon of reversible dissociation of native proteins, first uncovered by Svedberg's researches, of which there are many examples, including horse hemoglobin, the seed globulins, the hemocyanins and the erythrocruorins. Such dissociable proteins (which fall, it is claimed into aliquot parts), break up into smaller units which still have the protein character. They are therefore more properly called protein particles. That the stability of a protein unit or of a protein particle or colony of protein units can be ensured by linking of protein units together instead of the linking of each with a full complement of waters or other "foreign" molecules is shown in such a case as horse hemoglobin, where plain dilution with water causes the presumably dimeric molecule present when the concentration is as high as one per cent to be replaced by (half-sized) monomeric molecules when the concentration is below 0.5 per cent. Dissociations of other proteins by dilution with many other molecular species of polar molecules, including arginine, lysine and so on are also known to occur.

The picture of the proteins as genuine molecules, whether monomeric or polymeric, which comes from these physico-chemical studies is amply confirmed by crystallographic studies which

paint the same picture, with even greater sureness. These studies have shown that native protein molecules have definite atomic patterns. The X-ray pictures of horse hemoglobin are of such perfection that there can be no doubt that each molecule consists of a highly organized framework of atoms. Further, the transition from the "wet" to the "dry" crystals of this and other proteins indicates that some or all the R-groups of the residues, although rooted in definite spatial patterns on or in skeleton frameworks (whether of surface or volume type), are capable of considerable elasticity and flexibility.

The immunological picture is entirely in accord. Marrack, in fact, goes so far as to picture certain constellations of R-groups in definite spatial arrangements dotted about on the rigid surface of the native protein as the most natural interpretation of the immunological facts. How otherwise, indeed, can we interpret the subtle differences in the affinities of antibodies formed by an antigen formed from 1-phenyl-2, 3-dimethyl-4-amino-5-pyrazolon diazotized and coupled with protein and that formed when the haptene changes the position of a double bond.

The deduction by Marrack that surfaces of proteins carry R-groups in definite patterns leads us to another aspect of the matter which is perhaps one of the most crucial of all for biological structure problems. Evidently we have to assume that the patterns on one patch of a native protein, or shall we not rather say one face (since the surface as a whole must have a definite morphology which even in the most general case must be polyhedral in type) fits with certain faces of certain other proteins but by no means with any face of any protein. Thus specificity in protein surfaces leads naturally to specificity in interlinks and interlinking will in general be by means of R-groups located on this or that face of each unit. On this basis, protein units may be expected to form protein particles (as indeed Svedberg has already found in many cases) and structure systems of all degrees of complexity as T. Brailsford Robertson (in 1918) long ago suggested. Such structure systems in the present picture will consist of rigid skeletons held together by interlinking groups. One main feature of all the biologically-functioning systems we are considering, namely their flexibility, is now at once interpretable. A molecule such as methane consists of atoms held in definite directions and at definite distances, allowing very little flexibility within the molecule. But an association of protein units into a protein particle or framework is of a totally different type so far as its mechanical properties are concerned. Any interlinking of protein units by means of R-groups allows some flexibility of the resulting structure. This result offers a reasonable and straight-forward interpretation of the deep-lying dichotomy found in cytoplasmic structure. On the one hand, this picture of the nature of association of proteins shows that it will be extremely specific, in so far as it involves the compatibility of patterns of R-groups. Association, being dependent upon R-group constellations fitting into each other sufficiently well to form a stable combination, is necessarily conditional upon the maintenance intact of the characteristic skeleton, i.e., upon the condition that the native protein structure is preserved. Yet, on the other hand, such associations of protein units will give systems with definite flexibility. And this flexibility, let it be noted, is conjoined with and not in any sense incompatible with extreme specificity which is inherent not only in the individual units and their disengaged faces, if any, but also in the pattern of the various interlink types in the system as a whole. Putting these two superficially incompatible characteristics of specificity plus rigidity of the skeletons and flexibility of the R-groups together we have a unit which promises well as the chief "structure-forming" ingredient in biological structure systems.

Geometry Will See Us Through

From a totally different field of work, my own field of mathematics, there issues the same picture. This synthetic-geometrical attack has excited so much controversy from such able critics, con and pro, that there seems little doubt that, even if some of the arguments in favor of the theory may have so far been missed, practically all the objections which can be brought against it have been meticuously tabled, a great advantage for any who might wish impartially to assess the situation regarding native protein structure. On examination (see Langmuir, *Proc. Phys. Soc. London, 51,* 592, 1939; Wrinch, *J.A.C.S., 63,* 330, 1941) these objections seem to be lacking in force or cogency. Neither do they gain in these respects from constant repetition by those who thought them up, or by interested bystanders or workers in totally different fields, who didn't. Perhaps the most tenuous of the attempted onslaughts is that having to do with bond energies (known to be variable in different atomic environments) which might be possessed by a structure which has never been synthesized (so that no thermal measurements on it can be made) and their relation to a few thermal measurements on a single protein (denatured trypsin) whose structure is totally unknown. But the point which concerns us particularly in this new field is that the objections have focused attention upon details of the particular "fine structure" in terms of which the general idea of closed fabric structures for the globular proteins was clothed for expository, exploratory and general mathematical purposes. The actual fine structure or atomic pattern is, of course, quite in-

susceptible of proof or disproof by gross chemical techniques in view of the lability of the native protein. X-ray analysis at first sight appeared to offer some hope of clinching the matter, and indeed it was shown by Langmuir and myself that the predicted structure for insulin is compatible with the only X-ray data available. However in view of the subsequent pronouncement by a distinguished X-ray authority that these data are too poor to be used for this purpose, this aspect of the matter is held up for the moment until data which these experts consider of value shall be available to test each and all of the predicted structures.

I have stated that this concentration of the interest of my distinguished critics and others on the "fine structure" aspect of the work is of special interest to us here and now, for the following reason. The essential point of the work has, apparently, been entirely missed and it is this which is relevant to the protein structure systems which we now have under consideration, namely the possibility that the essence of these large structural units of proteins lies in a characteristic atomic fabric bent around to form a closed surface, a suggestion which could have a powerful influence in the design of experiments. The emergence from the fields of physical chemistry, protein crystallography and immunology of the genuine megamolecule, a structure consisting of thousands or millions of atoms which yet possesses definite physical, chemical and above all specific biological properties poses a new problem to physics and chemistry. The crisis is resolved and a beautiful new field of work is opened when it is seen that any patterned fabric of atoms gives the clue to this phenomenon. For any fabric of atoms will by its own pattern determine the types of closed surface which the metrical requirements of its constituent atoms will allow it to build. If the pattern of the fabric is a small pattern, then the resulting closed structures will give molecules of low molecular weight, like hexamethylene tetramine. But if the building units essentially require a large repeat in the pattern, as is the case with amino-acid and imino-acid residues, then the molecular weights will be large. But, whether the molecules are large or small, the number of atoms or units in the skeletons will be determinate and a reason is then seen why even enormous protein structures should consist of perfectly definite numbers of residue units. Indeed we can see one step further and predict that appropriate residue numbers for protein skeletons should fall into definite series, necessarily quadratic functions of the natural numbers, since we are concerned solely with surfaces, i.e., two-dimensional distributions. It is on this basis that atomic fabrics which have been suggested in my investigations have yielded 72 as the first skeleton residue number, 288 as the second and so on, as the residue numbers for native protein units and multiples of any of these for protein particles, numbers which accord well with the molecular weights found by Svedberg.

There are other striking consequences of this very simple idea. In a sense there is, as our colleague in China, Dr. Wu, was perhaps the first to emphasize, some general structural character which may be regarded as the essence of the protein character. Yet there are astronomically many different proteins, all made up of a strictly limited variety of building stones. The cage or perhaps some other closed fabric structure like an anchor ring, with its obvious significance in terms of denaturation, according to which any breakdown of the closed structure destroys the native character, may be, I submit, the essential protein character. So long and only so long as the R-groups have their roots held in this spatial pattern, is the specificity of the molecule retained. The ways in which the fixed number of places for residues in this highly organized, multiply-connected atomic skeleton can be filled by different complements of the residue varieties, or even by the same complement, are, very evidently, extremely large in number. Each and every pattern of arrangement connotes potentially one very highly individualized and specific native protein unit. It is plain on this theory how the same skeleton can be used for the construction of native proteins with very diverse physical or chemical properties. Thus a skeleton having some definite number of residues may give rise to a molecular weight class—necessarily of wide spread, if anything like the available varieties of individual residue weights is made use of—containing proteins entirely distinct in their biological functions and chemical and physical identities. This situation is in fact realized in Svedberg's molecular weight class at about 36,000 for which the residue number of 288 was predicted in 1936 on the basis of the cyclol theory. This class contains lactoglobulin, pepsin, insulin, chymotrypsin and a number of other entirely distinct protein species.

A further aspect of the work requires a word of comment, in view of the necessity of relating any proposed structures to known facts regarding the high capacities and low conductivities of some biological structures. I refer to the fact that the fabric cages, which are here suggested, break down into monolayers with the utmost ease, (indicating once again the dependence of their stability on interlinks with other molecules, water in this case) and to the possibility that the prodigious insolubility of these monolayers may indicate that the native protein had in its interior a considerable complement of hydrophobic R-groups, shielded by the skeleton from contacts with a watery world.

(Concluded in Next Issue)

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris I. Gorokhoff and Judy Woodring.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879, and re-entered, July 23, 1938.

NOMINATIONS FOR TRUSTEES

The Annual Meeting of the Corporation of the Marine Biological Laboratory will be held in the auditorium of the Laboratory on Tuesday, August 12, at 11:30 A.M., for the election of Officers and Trustees and the transaction of other business. The Trustees will convene the same morning before the Corporation meeting and again in the afternoon.

The Nominating Committee of the Corporation of the Marine Biological Laboratory has posted the following slate:

For Trustee Emeritus—W. J. V. Osterhout

For Treasurer of the Corporation — Lawrason Riggs, Jr.

For Clerk of the Corporation — Philip B. Armstrong

For Trustees of the Class of 1945—W. R. Amberson, S. C. Brooks, W. C. Curtis, H. B. Goodrich, I. F. Lewis, R. S. Lillie, A. C. Redfield, C. C. Speidel

For Trustee of the Class of 1943 to replace W. J. V. Osterhout—G. H. A. Clowes

For Membership in Executive Committee—D. E. S. Brown, B. H. Willier

Drs. Clowes and Brooks are proposed for Trusteeship for the first time; the other seven men are presented for reelection.

ADDITIONAL INVESTIGATORS

Addison, W. H. F. prof. normal histol. & emb. Pennsylvania Med.
Auger, C. Rockefeller Inst. Br 206.
Bongiovanni, A. M. grad. biol. Villanova. Rock 3.
Bowser, E. R., Jr. asst. fel. biol. Pittsburgh. Rock 7.
Bowen, E. prof. biol. Gettysburg (Pa.).
Deyrup, Ingrith grad. zool. Columbia. OM Phys.
Green, J. W. grad. phys. Pennsylvania. Br 205.
Gudernatsch, F. prof. biol. New York. Br 344.
Kaylor, C. T. instr. anat. Syracuse Med. Br 226.
Magnus-Levy, A. res. assoc. Yale. lib.
Loewi, O. res. prof. pharmacol. New York Med. lib.
Maurer, Jane techn. Yale Med. Br 109.
Meed, Margaret R. grad. biol. Brown. Br 312.
Memhard, A. R. (Riverside, Conn.). Br 334.
Nash, J. F. grad. asst. New York Med. Br 234.
Oppenheimer, Jane M. instr. biol. Bryn Mawr. Br 118.
Ormsbee, R. A. grad. asst. Brown. Br 331. Ka 21.
Packard, C. asst. prof. zool. Columbia. Br 102.
Perkins, Patricia J. grad. asst. chem. Cincinnati. OM Phys. WF.
Rollason, H. D., Jr. grad. asst. biol. Williams. OM Phys. Dr 7.
Sevag, M. G. asst. prof. biochem. Pennsylvania Med.

DATES OF LEAVING OF INVESTIGATORS

Costello, D. P.	Aug. 6	Mitchell, P. H.	Aug. 2
Forbes, J.	Aug. 2	Mullins, C. P.	July 29
Hager, R. P.	July 31	Runk, B. F. D.	July 26
Henry, R.	Aug. 2	Schaffel, M.	July 29
Hunninen, A. V.	Aug. 9	Spratt, N. T., Jr.	July 29
Levin, E.	July 29	Warner, E. N.	July 25

REPRESENTATION BY INSTITUTIONS AT THE M. B. L.

The following institutions are represented by three or more investigators registered (filled out registration blanks before August 8) at the Marine Biological Laboratory this summer:

Institution	1941	1940
Pennsylvania	30	34
New York	23	21
Columbia	18	22
Yale	13	8
Hopkins	12	8
Harvard	9	7
Ohio State	9	8
Chicago	8	13
Rockefeller Institute	8	9
Michigan	7	4
Princeton	7	5
Brown	6	5
Cornell	6	6
Pittsburgh	6	7
Cincinnati	5	4
Lilly Laboratories	5	4
Milton Academy	5	4
Vassar	5	2
Villanova	5	2
Washington	5	5
California	4	5
California Tech.	4	2
Iowa	4	4
Mt. Holyoke	4	2
Queens	4	4
Rochester	4	2
C. C. N. Y.	3	3
Illinois	3	2
Miami	3	2
Minnesota	3	2
Missouri	3	6
Oberlin	3	3
Syracuse	3	5
Temple	3	3
Toronto	3	5
Union	3	4
Williams	3	3

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
August 10	6:34	6:54
August 11	7:18	7:40
August 12	8:01	8:27
August 13	8:47	9:15
August 14	9:35	10:08
August 15	10:26	11:02
August 16	11:19	11:58
August 17		12:13

In each case the current changes approximately six hours later and runs from the Sound to the Bay.

ITEMS OF INTEREST

Dr. W. E. Martin, who is instructing in the invertebrate course, has been promoted from assistant to associate professor of zoology at DePauw University.

Dr. Edgar J. Boell, who worked here last summer, has been promoted from assistant to associate professor of biology at Yale University.

Dr. Frank H. Johnson, instructor of biology at Princeton University, has been promoted to assistant professor.

Dr. John O. Hutchens, who has been a graduate student in zoology at the Johns Hopkins University, has been appointed instructor in physiology at the University of Chicago.

A Research Institute of Endocrinology has been established at McGill University. Dr. J. B. Collip, who has been chairman of the department of biochemistry, will relinquish his position to become director of this Institute.

On August 1, there were 278 investigators present at the Marine Biological Laboratory, as compared with 293 at the same time last year.

The Trustees of the Woods Hole Oceanographic Institution will hold their annual meeting on Thursday, the fourteenth of August.

The second edition of Dr. James Mavor's textbook, "General Biology," was published in June. Dr. Mavor is professor of biology at Union College and is now working at the Marine Biological Laboratory.

At the staff meeting of the Oceanographic Institution on Thursday evening, Dr. Alfred C. Redfield spoke on "The Distribution of Nutrient Substances in the Ocean."

An informal statistical seminar will meet twice weekly during August in the Smoking Room at the Fisheries Residence with Dr. C. I. Bliss in charge. The first meeting was held on Thursday. This summer the design of experiments will be emphasized, but the program can be adjusted to fit the needs of those attending.

M.B.L. CLUB NOTES

In the ping-pong tournament, competition is planned in men's singles, women's singles, and mixed doubles, and perhaps in men's doubles and women's doubles. Persons wishing to compete should sign up on the chart at the Club House or see Teru Hayashi.

The following composition will be presented at the phonograph record concert at the M.B.L. Club on Monday evening: Beethoven, "Missa Solemnis" in D (Opus 123), performed by the Boston Symphony Orchestra, conducted by Dr. S. Koussevitsky, with the Harvard Glee Club and the Radcliffe Choral Society.

Professor Otto Loewi, who shared the Nobel Prize in 1936, arrived in Woods Hole last week with Mrs. Loewi. They will spend the balance of the summer here.

Dr. James E. Kindred, professor of anatomy at the University of Virginia, who has worked here for a number of years, is supervising the building of a home in Charlottesville, Virginia, this summer.

Dr. David Hand, assistant professor of biochemistry at Cornell University recently visited Woods Hole with Mrs. Hand on their 32-foot auxiliary cruiser *Camara*.

Miss Helen S. Morris, teacher of biology at Evander Childs High School, in New York City, visited Woods Hole recently. She took the course in botany in 1927 and returned for research work for several summers afterwards.

Mrs. Mildred W. S. Schram, executive secretary of the International Cancer Foundation, who worked at the Marine Biological Laboratory last year, is spending this summer at the Mt. Desert Biological Laboratory at Salsbury Cove, Maine.

Dr. M. Perrot is working this summer at the Biological Laboratory at Cold Spring Harbor. During the academic year, he teaches at the University of Missouri.

Dr. Linville A. Baker was called to Annapolis at the end of last week to take an ability test for second lieutenant in the Medical Administrative Corps Reserve. He was away three days.

Miss Grace Workman, who was here last year, was recently married to Dr. John W. Scott, of the Toronto General Hospital staff. Dr. Scott took the physiology course at the Laboratory in 1939.

The *Atlantis* sailed on Wednesday on one of its regular cruises to the Gulf Stream under the direction of Dr. Alfred Woodcock. She is expected to return in four weeks.

M.B.L. TENNIS CLUB NOTES

Play for the tournaments is now under way. Competition is going on in the men's and women's singles, and mixed doubles classes; the finals in these classes are planned for Saturday, August 16. The entries in the men's doubles are being held open for an additional week; if eight teams sign up this class will also be included in the tournaments.

The annual meeting will be held in the Old Lecture Hall next Wednesday at 7:00. A proposal to amend the by-laws to permit the members of the immediate families of M.B.L. Corporation members to hold office will be considered.

INVERTEBRATE CLASS NOTES

With our second week at Woods Hole there has come a clearer understanding of the resources of the place, of how best to take advantage of them. In the lab one cannot examine all the material available; with the aid of the lectures and abundant mimeographed directions one can select material of particular interest. Our work on Coelenterates included a study of the transparent sea anemone *Nematostella*, much of whose internal structure is discernable without dissection. This ideal lab specimen with very limited geographical distribution (Woods Hole, the Isle of Wight) was found in the Eel Pond three years ago by Dr. Bowen. The beautiful Ctenophore, *Mnemeopsis*, was also available.

Our work with Platyhelminthes has revealed no beautiful specimens comparable to the comb-jelly, but Dr. Rankin has launched on a speedy efficient three-day defense of his pets, his accounts of weird life cycles being confirmed by our own lab studies.

Saturday's collecting trip to Lagoon Bridge on Martha's Vineyard provided an excellent antidote to a week spent in the lab. The somewhat dubious weather made up its mind, by the time we got there, in our favor. How time flew as we scraped piles, passed mud and sand through the sieve, and turned over rocks! The photographers among us were particularly busy during the picnic lunch on the beach. We got back to work before we had a chance to feel lazy, to be interrupted all too soon two hours later by the whistle announcing departure. So great was the force of the flowing tide that one of the boats was unable to point its bow into it, remaining hard against the bridge piles. By dint of much churning and a tow, we finally got headed for a sunny trip home. The trip was not complete, of course, until the specimens were placed in clean water, examined and labeled.

There were few people to be seen in the lab Monday evening. On this occasion, the hard hitting invertebrate team, paced by Bob Corder's home run in the first inning, trounced the highly touted crew team, ten to two. Much credit is due to the four-hit pitching of the Wabash star Howie Miner. While the crew was popping flies, the "Invert" Sluggers were busy collecting their sixteen hits.

Conspicuous feature of the crew's game was the sparkling and vociferous play of catcher Jasper Trinkhaus. With the restoration of their original line-up, the crew expect to "clean the bases like (they) clean the lab."

Not all the talk in the lab is of worms. There is proud papa Hauschka's delight in his 16-month old child's unmistakable flair for biology which has manifested itself in the latter's eating algae on the beach. It is now an acknowledged fact that Ann Weber was 1940's champion woman archer. They will never stop twitting Howie Miner about the letter he received from Gypsy Rose Lee. Arthur Culbertson injured his knee rushing to the dock to get a better glimpse of some interesting vertebrates on board an outgoing boat. Six members of the class, none of them expert seamen, set out Sunday morning in one of Hilton's catboats. As they tacked back and forth they noticed squashes floating with the tide. It soon became apparent there was but one squash; with all the maneuvering they scarcely kept up with the drifting vegetable. After a picnic lunch they commenced their return trip, riding the tide the other way. Just in sight of Woods Hole, one canny mariner discovered the wake was off the *bow!* To make a long trip short, the suspecting Hilton arrived at 8 P. M. and towed them home.

—Louise Gross and Bill Batchelor

BOOK REVIEW

JOHANNSEN, O. A. and F. H. BUTT. "Embryology of Insects and Myriapods". pp. xi + 462. New York, McGraw-Hill Book Company. 1941. $5.00.

Periodically a work of real scholarship emerges from the welter of printed matter with which the scientific world is annually presented. That the present volume should fall under this classification is the more remarkable in that it is stated to be "the outgrowth of a course of lectures"; most such outgrowths are either pathological, being excised under the scalpel of the publisher's auditor, or insignificant warts which succumb to the caustic of public opinion. Johannsen and Butt are here presenting a source book on arthropod embryology which has reached, in its first printed edition, a degree of excellence which shows clearly that it has passed through many editions in manuscript.

Two choices, either to write a clear account of the subject or to present both sides of all questions, confront the author of a text of this nature; these choices form a dilemma on which has been caught many a promising volume. Johannsen and Butt avoid this impalement by attacking each horn separately. Their work is divided into two parts, the first ostensibly dealing with embryology under its biological subdivisions, and the second with the same subject under its taxonomic subdivisions. The first part, however, gives a clear, well documented account of what appears to them to be the most probably true story. Doubtful points are cross referenced to the second part in which some 800 references have been reduced to a coherent and succint summary. Three hundred and seventy well-drawn and clearly labelled fig-

ures enrich the text.

The reviewer, who is after all expected to criticise as well as praise, cannot refrain from one grumble. Chapter XII, which is called "Experimental Embryology", does not belong in a book of this general excellence. The authors appear to have been misled in their selection of material by considerations of what would appear most interesting to a vertebrate embryologist. Such purely entomological experiments as pupa grafting, transplantation of possible endocrine organs and the discovery of a pupal cuticle softening gland in certain coleoptera, have been ignored in favor of the few experiments which have been done on determination and organising centres.

The publishers are to be congratulated on maintaining the high standard of production which has marked their previous publications in the zoological sciences. *Peter Gray*

EFFECT OF AZIDE ON *CYPRIDINA* LUCIFERIN

(Continued from Page 121)

measured with an apparatus similar to that described by Anderson (*J. Cell. Comp. Physiol.*, 1933), involving a photoelectric cell, condenser, Lindemann electrometer, and potentiometer.

There are a number of data now available which bear on the chemical structure of luciferin. First, it is definitely not protein and probably not lipoid, as indicated by its solubility characteristics (Anderson, *J. Cell. Comp. Physiol.*, 1936). Anderson has also called attention to the fact that its oxidation-reduction potential is similar to those of certain naturally occurring polyhydroxy benzene derivatives studied by Ball and Chen (*J. Biol. Chem.*, 1933). The absorption spectrum of luciferin solutions (Chase and Giese, *J. Cell. Comp. Physiol.*, 1940) bears a marked qualitative resemblance to those of certain naphtha- and anthraquinone derivatives measured by Morton and Earlam (*J. Chem. Soc.*, 1941). The visible absorption spectrum of luciferin solutions undergoes changes during oxidation which may also indicate a quinoid structure (Chase, *J. Cell. Comp. Physiol.*, 1940). Finally, Giese and Chase (*J. Cell. Comp. Physiol.*, 1940) found that cyanide combines irreversibly with purified luciferin to give a compound incapable of luminescence when luciferase is subsequently added. They interpreted these results as indicating an aldehyde or keto group in the luciferin molecule as the point at which cyanide, and probably luciferase, acts.

Chakravorty and Ballentine (*J. Amer. Chem. Soc.*, 1941) have summarized most of the chemical data on luciferin and, on the basis of these and of certain additional experiments of their own, have suggested a partial structure for the luciferin molecule, consisting of a hydroquinone nucleus and a keto-hydroxy side chain. The keto group in the side chain would be the site of action of cyanide and of luciferase, the hydroxy group would be the point of action of benzoyl chloride and other similar reagents, and the hydroquinone nucleus would be capable of reversible oxidation and would thus represent this characteristic of luciferin.

Giese and Fisher (unpublished observations) have found that sodium azide markedly inhibits the luminescence of luminous bacteria and this observation prompted the present experiments on effects of azide on the luminescent reaction between *Cypridina* luciferin and luciferase. At pH 6.6 sodium azide, in concentrations from 0.01 to 0.1 M., inhibits the luminescent reaction reversibly and in such a way that a combination between the azide and the luciferin is suggested. At pH 5.4 a smaller concentration of sodium azide produces the same degree of inhibition of luminescence. Comparison of results at these two pH's indicates that it is the undissociated HN_3 which is effective, rather than the NaN_3. When the data are tested in terms of the mass law equation by plotting logarithm of percent total luminescence obtained minus logarithm of percent total luminescence inhibited against logarithm of sodium azide concentration, they are found to be fitted by straight lines with slopes approximately equal to unity. This might indicate that a single azide molecule or ion combines with a luciferin molecule to produce the observed effect.

The following interpretation of these results in terms of the supposed structure of the luciferin molecule is intended as purely tentative. Schmidt (*Chem. Abstr.*, vol. 19, p. 3248), Franklin (*J. Amer. Chem. Soc.*, 1934), and others have described the effect of hydrazoic acid on certain alcohols, aldehydes and ketones, for example, but the conditions of the reaction are very drastic compared with those of the present case and it seems unlikely that such a reaction would occur, even though the experiments with cyanide have indicated that an aldehyde or keto group may be present in the luciferin molecule. Fieser and Hartwell (*J. Amer. Chem. Soc.*, 1935) have reported the action of hydrazoic acid on benzo- and naphthaquinones. An azido-hydroquinone is first formed and this may change to an amino-quinone with liberation of nitrogen. This type of reaction does not seem to require very drastic methods and, in view of the oxidation-reduction potential of the luciferin system and the absorption spectrum of purified luciferin solutions, it seems possible tentatively to interpret the effect of azide on luciferin as indicating a reaction with a quinoid structure.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on July 29.)

THE COLLECTING NET

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INTERPRETATIONS OF EFFECTS OF CO AND CN ON OXIDATIONS IN CELLS

DR. MATILDA M. BROOKS

Research Associate, University of California

I. *Effects on Rate of Oxygen Consumption.*

In these preliminary experiments, eight stages in the development of sea urchin or starfish eggs were used to see what effects certain accelerators or inhibitors of O_2 consumption would have. The stages were unfertilized eggs, first cleavages, 32-cell to morula, blastula, early gastrula, late gastrula, early pluteus, and late pluteus in the case of *Arbacia,* and unfertilized eggs and first cleavages in the case of *Asterias.* Methylene blue which is assumed to be an accelerator and KCN and CO which are assumed to be inhibitors of respiration were used. The experiments were done in triplicate by the Warburg method using aliquot portions of a suspension of eggs or larvae in sea water for each of the 12 Warburg vessels operated simultaneously. Concentration of methylene blue was .00012 M; of KCN, .00025 M and of CO as near to 100% as was possible to obtain.

It was found that (*Continued on page* 150)

M. B. L. Calendar

MONDAY, August 18, 8:00 P. M.
Lecture: Dr. R. W. Wilhelmi: "Serology Applied to Problems Involving Phylogenetic Relationships and Evolution of Animals."

TUESDAY, August 19, 8:00 P. M.
Seminar: Dr. D. Nachmansohn: "Electrical Potential and Activity of Choline Esterase in Nerves"; Dr. A. Claude: "Chemical Composition of Mitochondria and Secretory Granules"; Dr. D. Wrinch: "Native Proteins and the Structure of Cytoplasm."

THURSDAY, August 21, 8:00 P.M.
Lecture: Dr. H. Dam: "The Biological Significance of Vitamin K."

FRIDAY, August 22, 8:00 P. M.
Lecture: Dr. O. Meyerhof: "Nature, Function and Distribution of Phosphogens in the Animal Kingdom."

THE ORIGIN OF GAPS BETWEEN SPECIES

DR. ERNST MAYR

American Museum of Natural History, New York, N. Y.

Some day, when the history of the science of evolution will be written up, the historian will have to distinguish various periods. The fact that we seem to be right at the beginning of a new one of such periods makes the work in the field of evolution so particularly interesting. Up to Darwin, evolution was, more or less, a speculative subject. The publication of "The Origin of Species" in 1859 started a second period in the history of the science of evolution. In this period all the heavy artillery of the biological disciplines of paleontology, taxonomy, comparative anatomy and embryology was assembled to demolish any doubts and objections that were raised against the principle of evolution. Final victory of this battle was gained by the end of the century and the discussion moved from the question: Is there evolution? to the question: How does evolution proceed? This is the question on which the student of evolution

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THE MARINE BIOLOGICAL LABORATORY

has concentrated since 1900.

Darwin entitled his epoch making work not the "Principles of evolution" or the "Origin and development of organisms." No, he clearly realized that the species problem was the core of the problem of evolution, and he, therefore, called his book boldly "On the Origin of Species." This is a fact that should not be forgotten.

Speciation is the name we give to an evolutionary process by which *one* former species is broken up into several separate units which have changed sufficiently to be considered different species. There are thus actually two processes involved: the development of diversity as well as the establishment of discontinuities between the newly evolving species.

The development of diversity is the more obvious one of these two aspects of evolution and the work of the geneticists between 1900 and 1930 was primarily devoted to an elucidation of this aspect. This work consisted in an analysis of the differences between various species and other natural units, and in an investigation of the genetic basis of these differences and of the origin of new variants, the so-called mutations. Some phases of this work are still incomplete, in particular what we might call the physiology of mutation and gene action, but the major principles of chromosomal inheritance and mutation are now well established and generally accepted.

However, those geneticists who were most interested in the evolutionary aspects of genetics, men like Dobzhansky and Sewall Wright, were the first to realize that even if we knew everything about variation and mutation, we would still be far from an understanding of the origin of species. After all, it is quite thinkable that such variation would only lead to a single interbreeding community of immensely variable individuals. But this is not what we find in nature.

The taxonomist, who approaches the diversity of organic life, finds that it is not one homogeneous mass of genetically different individuals, but rather that it can be broken up into smaller, natural units, the so-called taxonomic categories of families, genera, species, etc. Each one of these is more or less separated from the other categories; they form a series of discontinuities. Evolution is a continuous process, but the units produced by evolution are discontinuous.

I have already said that we seem to be at the beginning of a new phase of the study of evolution and what I meant by this phase is the study of the origin of discontinuities. This is a typical border line field which must be studied jointly by the naturalist and ecologist, by the geneticist and taxonomist.

Now let us study the question of interspecific discontinuities as exemplified in some of the familiar New England birds. The ornithologist unites for example, the thrushes in the genus *Hylocichla*. But if we examine the variation within this genus in more detail we find that it clusters very closely around five means to which we apply the familiar names wood thrush, veery, hermit thrush, gray-cheeked thrush and olive backed thrush. They are all quite similar to each other, but two or three of them may nest in the same wood without the slightest intergradation. Not a single hybrid is known between these five common species.

To take another example: if we sail out to the tern islands of the New England coast, we will find the three species: the common tern, the roseate tern, and the Arctic tern. All three species again are very similar, so similar indeed that only an expert can tell them apart in the field, but there is not a single intergrade or hybrid known between the three species. Moreover, they differ in their ecology and behavior patterns. In all these cases the related species are separated by clearcut discontinuities or what Goldschmidt would call "bridgeless gaps."

Such observations lead us to the assumption that the species is a basic and objective natural unit. The majority of the animal taxonomists, particularly of the better known groups, subscribe to this opinion. But there is a considerable minority of authors who say just exactly the opposite. To them, the individual is the only unit in nature which possesses any reality while species are merely abstractions. They claim that all organisms form a continuity, which the taxonomist chops up into species merely for the sake of convenience, to be able to handle them better in the museum drawers.

The real test of the question, whether species have an objective reality or not, can only be made in groups which are taxonomically well known. Birds are, unquestionably, such a group. Now if we ask ourselves, are species objective units in birds or not, we must ask at once, what are the criteria by which the objectivity of a systematic unit can be determined? Thinking this over, we come to the conclusion that such a unit is objective or real, if it is delimited against other units by fixed borders, by definite gaps.

Now we mentioned already that such definite gaps exist, if we compare all the New England thrushes or all the New England terns with each other. There is no question that they are good

The Collecting Net was entered as second-class matter July 11, 1935, at the Post Office at Woods Hole, Mass., under the Act of March 3, 1879, and was re-entered on July 23, 1938. It is devoted to the scientific work at marine biological laboratories. It is published weekly for ten weeks between July 1 and September 15 from Woods Hole, and is printed at The Darwin Press, New Bedford, Mass. Its editorial offices are situated in Woods Hole, Mass. Single copies, 30c by mail; subscription, $2.00.

species. The same is true, with few exceptions, whenever we compare species that occur at the same localities, what I call *sympatric* species. But there is a second type of relationship between species, namely between species that represent each other geographically, the so-called *allopatric* species. Let us look at a bird with which most of you are familiar, the junco, the little gray sparrow, with the white outer tail-feathers, that comes to our feeding stations during the cold season. This bird occurs in a number of populations in the Alleghanies from Georgia north and in the coniferous zone from New England to the Rocky Mountains and to the northern tree limit. All these populations merge into each other and even though the junco of the southern Alleghanies is slightly different from the Canadian bird, there is no doubt that it belongs to the same species. The trouble starts in the Rocky Mountains. Here we find a series of mountain forms which extend from Alaska south as far as Panama, and some of which are completely isolated from all other junco populations. Most of them, however, interbreed where they meet each other, even though some of these forms are so distinct that the taxonomist prefers to call them different species. Clearly these allopatric species are not separated from each other by bridgeless gaps; they can not be delimited in an objective way. Such a situation is, of course, not really surprising because it would be exceedingly difficult to understand evolution if we did not have such cases of incompletely separated species or incipient species. We have learned from this discussion that species can be delimited from two kinds of other species, from sympatric species, where the gap is complete, and from allopatric species, where the gap is frequently incomplete.

Let us now look a little more closely at this delimitation and begin with the simple situation of the gap between sympatric species. In well worked groups, such as birds, the taxonomist does not encounter any serious difficulties when he tries to delimit sympatric species against each other. If there are any difficulties the final analysis usually reveals that either (1) several stages or phases of a species are so different that they had been mistaken for different species or (2) just the opposite, that several species that occur in the same locality are so similar that they were considered stages of one species.

Illustrations for the first kind of difficulty are easy to find. They are, however, not of great theoretical importance. More interesting is the other class of difficulties where pairs or even larger groups of related species are so similar that they are generally considered as belonging to one species, until the final analysis clears up this mistake. A number of such recently analyzed species groups are known in the genus *Drosophila*, such as for example *Drosophila "obscura"* and *D. "affinis."* The species of the flycatcher genus *Empidonax* are the closest parallel to this that we can find among North American birds, although the analysis of this case was completed more than a generation ago.

"Sibling species," as I call these morphologically similar species, are common in some classes and orders of animals and rare in others. Their importance is the following: In the bygone days of a purely morphological species definition they were considered races of one species. And since many of these species were originally discovered on the basis of biological or ecological differences, they were originally established as biological races. The literature is full of references to "biological races" of this kind, but closer analysis showed in a great majority of the cases that these so-called "biological races" are perfectly good species on the basis of every single criterion except the morphological one. The sibling species around the Malaria mosquito, *Anopheles maculipennis,* are perhaps the most instructive illustration of this principle. All the various races described by earlier workers, such as *atroparvus, messeae, melanoon, sacharovi* differ not only in their ecology and habits, but also fail to interbreed in nature, and are completely or partially sterile if they are artificially crossed in the laboratory. A more painstaking analysis has recently revealed fairly constant morphological differences between some of these species.

I have discussed these sibling species in a little more detail because their existence has been quoted as evidence in support of the idea of sympatric speciation, a subject which we will presently examine more closely. Such an assumption is, however, not justified. There is much reason to believe that even in sibling species speciation proceeds by geographical variation.

Geographical variation

Let us now examine the gap that exists between allopatric, that is, geographically representative species. This can best be demonstrated by examining the distribution maps of some bird species such as *Pachycephala* and *Zosterops*. We can summarize these cases and the previously mentioned case of junco, by stating that we have in all these cases geographically representative forms which, although morphologically well characterized, are clearly descendents of one common ancestor and which have not yet lost their sexual affinity. This is proven by the fact of unlimited hybridization wherever such species ranges meet. Morphologically, such forms are species, biologically they are not. These groups of allopatric species are the *final* results of a process which we call geographical variation. Let us now examine the beginnings of this process.

If a number of populations of any widespread species are compared with each other, it is usually found that they show certain genetic differences. This has been proven abundantly by the work of Sumner and Dice on *Peromyscus,* of Goldschmidt on *Lymantria dispar,* of Dobzhansky, Timoféeff, Patterson, and others on *Drosophila,* to mention just a few of many similar investigations. The taxonomist finds the same in his work. Sometimes these differences are only slight differences of size, very often they are more pronounced.

Populations, with definite geographical ranges and fixed systematic characters, are called geographical races or subspecies. As long as these subspecies are distributed continuously as in the case of the mainland races of *Myiolestes,* there is no question that they all belong to the same species; they are one continuity. But wherever geographical or ecological factors produce barriers, we find allopatric species gaps and we must use our judgment if we want to decide whether or not we consider them species. This is, for example, true for the insular races of *Myiolestes.*

Such isolated populations are of the greatest importance for the question of evolution. When they first become isolated they may not be different at all from the parent population from which they split off. However, the three factors of mutation, difference of selective factors and differences of random gene loss will produce an increasing difference between the two populations. After some time the difference will be sufficiently large to be noticed by the taxonomist. He says the isolated population is a different subspecies. Eventually, this subspecies will be different enough to be called a different species by some taxonomists. But is it really a different species? Are there any criteria by which we can determine this? The old-fashioned taxonomist will say yes, there is a good criterion. If this isolated population is characterized by a well defined character which in its variation shows no overlap with the parent population, it is a species. But such a purely morphological species definition is not acceptable to the modern biologist, because species are, after all, not the creation of museum taxonomists, but products of nature.

We should, therefore, let nature make the decision what is to be considered a species, and not rely entirely on the subjective judgment of the taxonomist. Reproductive isolation or the lack of it, is nature's criterion. In other words, if two forms meet in nature and intergrade or freely interbreed, they must be considered as belonging to one species. If, on the other hand, two forms meet in nature and act like two different species, that is they do not interbreed, we must consider them as good species. A species definition based on these biological criteria would have to be formulated about as follows: "*A species consists of a group of populations which replace each other geographically or ecologically and of which the neighboring ones intergrade or interbreed wherever they are in contact, or which are potentially capable of doing so in those cases where contact is prevented by geographical or ecological barriers.*"

On the other hand, we do not include in the same species isolated populations that have changed to the point that they are no longer capable of interbreeding with the parent race. We are not concerned here with the question what kind of differences must develop between these isolated populations, to bring about this reproductive isolation. This is a question to be examined by the geneticist. The naturalist believes, and the majority of the geneticists endorse this idea, that the mere accumulation of genetic differences will eventually lead to a difference sufficient to prevent interbreeding.

What the taxonomist is interested in is the purely descriptive fact that the isolation of certain populations together with geographical variation will in due time lead to the formation of new species, in fact that this is the normal process of species formation. This is the concept held by the vast majority of the thinking taxonomists, but this point of view has by no means remained unchallenged. The most severe critic has probably been Professor Goldschmidt in his recent book "The Material Basis of Evolution."

Goldschmidt argues that the small variants which ordinary gene mutations produce are insufficient to account for the basic differences which exist between species. He, therefore, postulates that species must originate by revolutionary changes in the genetic make-up, his so-called systemic mutations, and not by a gradual accumulation of differences with the help of geographical isolation. It would lead too far, in this connection, to attempt a point by point refutation of Goldschmidt's argumentation. I will merely point out that Goldschmidt confuses completely the two kinds of gaps between species, namely between sympatric species and between allopatric species. The gaps between sympatric species are absolute or bridgeless as Goldschmidt says correctly, otherwise they would be no good species; the gaps between allopatric species are relative, otherwise there would not be any evolution.

Goldschmidt claims that geographical variation leads only to diversification within the species, but not to the formation of new species, as the taxonomist claims. Instead of demolishing Goldschmidt's arguments we shall try the more direct approach and marshal the evidence of the taxonomist in favor of the importance of geographical speciation. These arguments are:

(1) Every character that is known to separate good species has also been found on close study to

vary geographically. This concerns morphological and physiological characters and includes interspecific sterility. Far distant geographical races of some species may be partly or completely sterile with each other, while neighboring races are, of course, always completely fertile.

(2) There is no criterion by which it can be determined whether isolated forms, such as *Pachycephala, Bombus* and *Zosterops,* are subspecies or species. Geographical variation coupled with isolation, completely blurs the borderline.

(3) Well isolated forms may show extreme morphological specializations which under ordinary circumstances would be considered as of generic value.

(4) Isolation on islands leads to species formation as proven by double colonizations.

(5) Slight overlaps may occur after the breakdown of the isolating barriers.

(6) If the final links of a chain of races overlap, they may act like good species, even though connected by a series of intergrading populations.

All these cases prove that if a population of a species becomes isolated and remains large enough in this isolation, it may acquire characters in this isolation which may enable it to exist as separate species beside the parental species, by the time the isolation barrier has broken down. The discontinuity which had originally been artifically maintained by geographical barriers becomes physiological and reproductive and the one time single species has developed into two.

Occasionally it happens that the geographical barrier breaks down before the reproductive isolation has become completed. The result is extensive hybridization in the zone of contact. A celebrated case is that of the flickers.

Sympatric Speciation

I have treated speciation, up to this point, entirely from the point of view of the ornithologist. We know that in birds all incipient species have to go through the stage of geographical races. New species can develop only in geographical isolation. We might call this "geographical" or "allopatric" speciation. The question remains whether it is not possible in other animal groups to have speciation through *ecological specialization.* The discontinuity, which would eventually lead to a gap between good species, would have to develop, in such cases, within the population of one geographical district. We might call this: *sympatric speciation.*

Some authors believe that this type of speciation is very wide-spread. Careful recent studies have tended to disprove this assumption. Many cases that were formerly quoted as proof for sympatric speciation have now been unmasked as sibling species. Some authors forgot that sympatric speciation can operate only if some ecological device exists which makes the cross-mating of the two diverging lines impossible. Such isolating mechanisms are, for example, distinct and non-overlapping breeding seasons, or in the cases of parasitic or monophagous species strict host specificity with the mating taking place on the host. Enough such cases have been described to make me believe that sympatric speciation is of common occurrence in certain animal groups with very specific ecological requirements, but almost completely absent in all other animal groups.

Our knowledge on the process of speciation—that is, the establishment of discontinuous natural units—is still very obscure in many animal groups, for example in fresh water and marine forms. Considerable difficulties are also caused by the so-called cosmopolitan groups. The Cladocera, rotifers, tardigrades, to name just the most prominent ones, are groups that are composed primarily of cosmopolitan species. The same identical species may be found on every single continent and in every latitude from the Arctic to the Antarctic. This is not surprising if we remember that all these forms, or at least their eggs, can encyst. Such dry cysts can easily be carried by wind currents clear around the world. The difficulty for the student of speciation lies in the fact that the continuous swamping of every population by new immigrants does not permit the establishment of new forms, at least so it seems. No adequate explanation for speciation in these families and orders has yet been advanced. There are several possibilities. One is that there is actually no longer any speciation in the most successful and most widespread of these species. Speciation is restricted to the more localized and, from the point of view of dispersal less successful species. The other possibility is quite different. Most of the above mentioned groups (the tardigrades are a notable exception) have parthenogenetic generations sandwiched in between the bisexual ones. It is possible that a radically new mutation in a single individual affecting, for example, the mating behavior, can build up a sufficiently large population during the parthenogenetic period, to be able to survive afterwards as the ground stock of a new species.

I have mentioned this case merely to indicate that there are many unsolved problems in the field of speciation. The geneticists have contributed their share, by unraveling a good many of the problems concerning the diversification which leads to new species. However, the origin of discontinuities is a problem outside of this field, no matter how important it is in relation to genetics. And Dobzhansky says: "The aspect of discontinuity should be emphasized, not because it is the more important of the two [processes of speciation], but because it is the less obvious one to the superficial observer." Actually many of the

laboratory workers do not seem to understand the significance of the fact that the vast variability of nature is not continuous, but broken up into a hierarchy of separate groups, of which at least the species has an objective reality.

The ornithologists, and to some extent also the students of mammals, butterflies and beetles, have gone a long way toward understanding how the discontinuities develop which precede the origin of new species in their groups. It will now be the task of the students of other animal groups to investigate how far these findings are applicable in their own field, or else what additional evolutionary mechanism they can discover.

(This article is based upon a lecture delivered at the Marine Biological Laboratory on July 31.)

AGING PHENOMENA AND FACTORS INFLUENCING LONGEVITY IN *MACTRA* EGG CELLS

Dr. Victor Schechter
Assistant Professor of Biology, College of the City of New York

The unfertilized egg cells of the clam, *Mactra solidissima,* go through an interesting series of morphological changes with age. The most outstanding of these are, in time series:

(1) Indentation of the cortical region proceeds until the egg is grotesquely collapsed.

(2) Inability, upon insemination, to cast off polar bodies.

(3) Loss of ability to cleave.

(4) Lack of germinal vesicle maturation, upon insemination. Cortex still responds to sperm by partial rounding up.

(5) Spontaneous germinal vesicle breakdown at extreme age. Polar bodies occasionally formed. Cortex rounds up.

It is conceivable that these changes, on the whole, might be explained by a loss of osmotic materials within the egg, combined with a stiffening of the cortex. The final spontaneous germinal vesicle breakdown could be due to a release of salts, like calcium, from the egg cortex to the interior. This hypothesis suggests the experimental procedure of varying the concentration of salts in the medium.

The results are clear-cut, in showing:

(1) A prolongation of life in low calcium solutions.

(2) A lesser prolongation of life in high calcium solutions.

(3) The most marked prolongation in low calcium combined with high magnesium.

The anomalous effect of high calcium is difficult to explain in terms of the working hypothesis adopted above. Its effect might be to decrease permeability and retard the flow of materials across the cell membrane. The effect of magnesium can easily be thought of as due to the displacement of calcium in the egg cortex. It is also known that the breakdown of germinal vesicles by radiation is inhibited by magnesium.

There are other factors which are known to increase egg cell longevity. Among these are: acidity; KCN; low temperature; thyroxin; egg albumin; alcohol; glucose; gum arabic; reduced bacterial numbers.

That any of these are effective in any nutritive way seems ruled out by the facts that an activated egg cell will continue living without food at least through the embryonic stage; and an unfertilized egg cell will live for a longer period when placed in sterile sea water. The relationship of egg cell longevity to the presence or absence of bacteria is an interesting problem in itself. With regard to the other factors listed above it may be significant that several of them affect viscosity and with viscosity, pH, iso-electric points and salt binding power are colligative properties.

Although many interesting parallelisms may be drawn between growth, differentiation, maturation and senescence in the short pre- and longer post-fertilization life of an egg it would be unwise to attempt to extend to the compound animal body, as a whole, the findings with regard to the relationship of calcium to aging in egg cells. This is obviously because of the role of calcium in many functional and organ-forming processes. As a general cellular phenomenon the hypothesis is worthy of further investigation.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 5.)

PROTEINS IN ACTION

Dr. Dorothy Wrinch
Professor at Amherst, Smith and Mount Holyoke Colleges

(Continued from last issue)

Bonds, Bridges and Links

On the basis of this closed fabric structure for the native proteins, there seems then to be no difficulty in interpreting and integrating the findings from the three important experimental fields we have discussed. To see rather clearly what the implications are regarding the ways in which such structural units can build structure systems, it is then only necessary to study what is known, from structure chemistry, as to how the R-groups,

whose nature is well-known, may be expected to interlink or associate. There may, of course, be cases in which such interlinking is covalent in character, e.g., in the case of cystine residues or basic and acidic groups which have formed a peptide bond. There are, however, a considerable variety of other types which seem to be indicated. These include hydrogen bridges between glutamines and asparagines through their $CO\text{-}NH_2$ groups, similar interlinks between acidic and basic groups, e.g., in glutamic or aspartic acid and arginine and, most important for our biological problems, the bolting together of similarly ionized groups by means of divalent ions of opposite sign. It is well-known that hydrogen bridges have a definite energy, say 4 to 8 kcal./mole. Contrasting this with the energy in a carbon-carbon bond, which we may take as (say) 60 kcal./mole, we see that such bridges are essentially weaker links than covalent links. Even the simultaneous formation of (say) 6 would give a total energy of less than a single C-C bond. The nature of the treatments by which such protein particles as horse hemoglobin, the seed globulins, the hemocyanins and the erythrocruorins can be dissociated into smaller units and particularly their mildness suggest that weaker links of this polar type, rather than covalent bonds, may be the means of association in such cases.

An inventory of all the types of intermolecular association discovered in crystal analyses calls our attention also to a second type, which is still weaker, namely that due to van der Waals attractions between hydrocarbon groups, such as exist in hydrocarbon crystals. Such association will depend upon a favorable opposition of what we may call the van der Waals outlines of R-group constellations on opposed faces. While describing such a situation in terms of interlinks, it will be understood that the integrated effect cannot be analyzed into localized associations of individual atoms or links in the usual sense.

In this and other aspects, it is interesting to notice the similarities and dissimilarities between these two categories of association to be expected when protein units associate by means of their R-groups. Both types are properly called "weak", if they are considered in terms of individual pairs of R-groups and if our norm is the covalent bond, but a sufficient amount of simultaneously opposed groupings permits an association of considerable stability. Next, in both cases the specificity of the protein surfaces is the all-important point. While some measure of association may occur between almost any protein surface and another, since acidic and basic groups are present in most cases and non-polar groups can also stick together, it will only be possible to have a comparatively stable association when the particular constellations of many R-groups are favorable. This is, to my mind, the essence of the protein as a "structure-forming" component, to use Lillie's phrase. The specific pattern of the protein itself determines whether or not it will associate in stable combination with another specific protein or indeed with any other molecule, such as a sugar, or a phosphatide. This fact is, I think, at the root of the non-antigenic character of certain molecules in certain cases. This also is the essence of the formation of antibody-antigen complexes. It accounts, too, for the way in which certain molecules and ions can change the permeability of cell membranes. The very low order of magnitude of the number of molecules required in such cases is of great interest.

Specificity will also play a role in the van der Waals type of association. A highly organized structure like a sterol may form a comparatively stable association with the surface of a protein if the R-group constellation has an appropriate morphology. The difference between the two types of association resides in the higher stereochemical exigency imposed by the polar groupings. Crystal analyses have shown a comparatively small range of variation of the lengths and directions of hydrogen bridges. There is in fact a special character in hydrogen bridges, which is absent in the van der Waals type of association. We can picture methyl groups so arranged that those from one protein surface pack into pockets of methyl groups from any other protein surface and *vice versa*, by means of a kind of cushioning effect, such that the surfaces can be pressed rather closer together by means of the spreading of methyl groups, the methyl-methyl distance remaining substantially unchanged. But take a hydrogen bridge association in which (say) 9 or more glutamines from a face of one protein interlink with an equal number from the face of another protein and the situation is quite different. Several arrangements of the $-CH_2-$ groups, giving larger and smaller distances between the faces, might be possible, but the transition between the various states would not be a continuous one but rather a snapping into position. It is this last point which makes clear why, in a sense, protein structure systems will have pores which allow the passage of molecules of appropriately small size even though they are not lipoid soluble. Actual attempts to build structure systems in which protein units are interlinked by polar side chains will show that it is not possible to pack these hedgehog-like units together without interstices. There will inevitably be lacunae or pores surrounded by rigid boundaries of polar groups. In this way we can understand the free penetration of water, a phenomenon which has always proved difficult to account for, when models of membranes involving complete monolayers of lipoids are insisted upon. With molecules which penetrate through points in the framework where the association is of the van der Waals type the situation is quite different. In the

case of cushioning associations of this kind there will be no pores of definite size defined by rigid atomic boundaries. The penetration of molecules by means of nosing their way between non-polar groups will thus not be a question primarily of size, but far more a question of affinity to the groups in the surfaces of the cushions, i.e., of lipoid solubility. This picture is offered as a possible interpretation of the generalization regarding permeability of membranes in which Jacobs has summed up a large number of his own findings, according to which if molecules are sufficiently small they will enter the cell regardless of their lipoid solubility; whereas, if they are sufficiently lipoid-soluble they will enter the cell regardless of their size.

Even this slight preliminary discussion of permeability shows that the most significant essential points in the picture of structure systems of these native protein units is the implication regarding the varied nature of the interlinks. Just as the specificity of a native protein resides, on this theory, in the spatial patterns in which the various R-groups are rooted in precise positions in the rigid skeleton, so the specificity of a membrane, it is suggested, resides partly in these patterns on the skeletons and partly in the nature of the interlinks. With this picture, we can visualize systems which have wholly different specificities in virtue of different patterns of interlinking, even if there is little or no difference in the specificities of the constituent protein units. It follows that great differences in permeability by no means imply correspondingly great differences in the constituents of a membrane or cytoplasmic system. We are all familiar with the classical work of Dakin and Dudley which showed (I think for the first time) that chicken and duck albumin differ in their molecular structures, even though no difference in chemical composition could be found, a finding which points directly to spatial patterns of R-groups. As Parpart has pointed out, species differences as determined by permeability studies are not found to be correlatable with large differences in composition. Evidently, as he remarks, it is the molecular orientation which is the important factor, a suggestion I venture to interpret in terms of spatial patterns, not only of R-groups but also of interlinks. It is this gradually growing conviction among the experts in the field which finds in the present picture a fundamentally simple and straightforward interpretation in that the specificity of any protein structure system whatsoever must necessarily be a function not only of the specificities of its constituents but also of the specific patterns of the interlinkages.

The Protein in Modern Dress

We are then presented with the native protein in modern dress, a figure markedly akin, as I shall be able to show, to the picture in the minds of many biologists during the last eighty years. This protein unit is large, but of definite skeletal structure which gives it its protein character. It is rigid, so far as the skeleton is concerned, but it is covered by flexible R-groups. It may be that, so far as its skeleton is concerned, it exists in very few varieties of sizes, but each such protein genus, covered by this or that selection of the strictly limited number of different R-groups, can give rise to an astronomically large number of highly individualized protein species. Thus we see how the protein character can be conjoined with a variety sufficient to account for even so many proteins as would be formed were every suitable molecular species injected in turn into every species of living animal. Finally the native protein, this hedgehog-like structure consisting of hard kernel and softer bristles, depends for its stability on interlinking with other molecules: it cannot exist in isolation. Native proteins will hold native proteins by means of groups of bristles emerging from definite patches on the kernels and so preferentially in certain directions. Since the bristles are themselves of varied types and are rooted in a definite spatial pattern, whatever this may be, it will hold these molecules, on which its life depends, in specific fashion. Not any two faces of any two proteins will be capable of forming a stable association, since this depends upon the favorable arrangement of not one but many R-groups on each face. Cases may be expected in which protein faces not adapted to direct interlinking may be able to unite in a structure system by using as intermediaries molecules with affinities to the two faces. The possibility exists that the considerable complement of cephalin known to be present in erythrocyte systems and thought by many experts to be integral parts of the membrane structure may be playing just such a role as this, in view of the groups, both polar and non-polar, which are present in these molecules.

What then can we do with this native protein unit, if we wish to build structures possibly relevant to the nature of cytoplasm and cytoplasmic membranes? The answer is that we can build protein structure systems of a wide variety of types—wide enough I think to provide a starting point for a new *experimental* attack on the structure of cytoplasm and cytoplasmic membranes in general.

Thus we may start in the obvious way by considering systems of the three types, linear, areal and volume. A set of native protein united in a one dimensional array we shall call a molecular fibril, open when it is like the links of a chain, closed when it is like the beads in a necklace. (A molecular fibril or chain, which may be suggestive as a hypothesis in a variety of physiologi-

(Continued on page 148)

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris I. Gorokhoff and Judy Woodring.

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BIOLOGY AT THE UNIVERSITY OF TEXAS

The school year 1940-41 has been an eventful one for biological departments at the University of Texas.

Dr. T. S. Painter, professor of zoology and internationally known investigator on salivary gland chromosomes, was named Distinguished Professor by the University Board of Regents, in line with a recently adopted policy of giving recognition and increased remuneration to outstanding members of the regular staff. Dr. Painter is a member of the National Academy of Sciences and five other American scientific societies, has a long list of publications, and was one-time holder of the academy's Daniel Giraud Elliot Medal. He is starred in *American Men of Science*.

In January the botany and bacteriology department held its second annual statewide seven-day short course for health officers and laboratory technicians, under joint sponsorship with the State Department of Health. A week's work in University laboratories was opened to staff men from hospitals, clinics and county health units.

The Clayton Foundation of Houston awarded this year a $45,000 grant to the University to make possible an extensive study of the presence and behavior of vitamins in all kinds of living tissue. Dr. Roger J. Williams, University biochemist and discoverer of the vitamin, pantothenic acid, was placed in charge of the work. Named to assist Dr. Williams are Dr. Maxwell Pollack, formerly at Northwestern University and later employed by the Pittsburg Plate Glass Company of Barberton, Ohio, for research in synthetic resins; and Dr. Alfred Taylor, formerly assistant professor of zoology at Oregon State College.

The Clayton Foundation also made an extensive grant to establish a new laboratory to study brucellosis, a recurrent fever which blights dairy cattle, under direction of Dr. Vernon T. Schuhardt, University bacteriologist. Two Eastern experts are employed to assist Dr. Schuhardt—Dr. Norman B. McCullough of Detroit, Mich., and Leo Dick of Marshfield, Wisc., both former employees of Parke, Davis & Company.

A $34,520 grant has been received from the Rockefeller Foundation for three years of genetics research, under direction of Dr. J. T. Patterson, professor of zoology; Dr. Wilson Stone, associate professor of zoology; and Dr. A. B. Griffen, research associate in the University's Research Institute.

ADDITIONAL INVESTIGATORS

Burt, R. L. jr. res. fel. biol. Brown. OM 22. Ka 2.
Furth, J. assoc. prof. path. Cornell Med. Br 317.
Lucas, A. M. assoc. prof. zool. Iowa State. OM 29.
Velick, S. F. res. fel. biochem. Yale. Br 110.

DATES OF LEAVING OF INVESTIGATORS

Ballard W. W.	Aug. 14	Henry, R.	Aug. 2
Brooks, M. M.	Aug. 14	Horn, A. B.	Aug. 7
Bullock, T. H.	Aug. 15	Klotz, J. W.	Aug. 10
Costello, D. O.	Aug. 6	Mitchell, P. H.	Aug. 2
Dumm, Mary E.	Aug. 8	Plough, H. H.	Aug. 1
Dytche, Maryon	Aug. 7	Wolf, E. A.	Aug. 13
Forbes, J.	Aug. 2	Woodward, A.	Aug. 4
Gilman, L.	Aug. 11	Yntema, C. L.	Aug. 13

Dr. R. Ruggles Gates is rewriting this summer at the laboratory his book on "Biological Botany," which was to have been published by an English firm last fall. The typewritten manuscript, consisting of twenty-five chapters, together with drawings for 150 plates, was burned when the offices of the publishers were destroyed in an air raid on London last December. The handwritten manuscript, from which the typewritten one was made, had been mailed back to the United States, but was lost in transit. Chapters in the book included such topics as chlorophyll, photosynthesis, stomatal action, rare elements in metabolism, hormones, vitamins, viruses, X-ray patterns in relation to cellular structure, chromosome structure, etc., embodying much recent work.

Dr. John Buck, assistant professor of zoology at the University of Rochester, with his wife, Mrs. Elizabeth Mast Buck, is collecting and making experimental observations on fireflies in Jamaica this summer. His work is supported by a grant from the American Philosophical Society. The Bucks will sail from Jamaica on August 21. Dr. Gardner Lynn, associate professor of zoology at Johns Hopkins University, under the auspices of the Brooks Fund, is also on the island studying the development of frogs which have no free-swimming larval stage. With these two men are John Marbarger and James Dent, both of whom took the course in invertebrate zoology last summer. The group is working at an elevation of about 2,000 feet in the Blue Mountains of Jamaica in a mansion on a coffee plantation which has been converted into a laboratory and which has been provided for them by the museum at Kingston.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
August 17	.....	12:13
August 18	12:50	1:07
August 19	1:42	1:57
August 20	2:30	2:45
August 21	3:19	3:30
August 22	4:01	4:18
August 23	4:45	5:03

ITEMS OF INTEREST

At the meeting of the Corporation of the Marine Biological Laboratory on Tuesday Drs. G. H. A. Clowes, S. C. Brooks and Columbus Iselin were elected trustees of the Laboratory. A report of the meetings of the Board of Trustees and of the Corporation by Dr. Charles Packard will be printed in the next issue of The Collecting Net.

At the annual meeting of the Woods Hole Oceanographic Institution on Thursday, Dr. Vannevar Bush, president of the Carnegie Institution of Washington, was elected a Trustee to serve until 1944. The six Trustees whose terms ended this year were reelected. Among these is Dr. T. H. Morgan.

Dr. Edmund V. Cowdry, professor of cytology at the School of Medicine of Washington University in St. Louis, has been appointed head of the Department of Anatomy, succeeding Dr. Robert J. Terry.

Dr. R. W. Gerard has been promoted from associate professor to professor of physiology at the University of Chicago.

Dr. N. T. Mattox, who is instructing in the Invertebrate course at the Marine Biological Laboratory, has been promoted from instructor to assistant professor of biology at Miami University, Oxford, Ohio.

Dr. Carl Herget, who worked at the Marine Biological Laboratory two years ago, has been appointed instructor in physiology at Cornell University Medical College.

Miss Dorothy Dole, who graduated from Bates College in June and who is taking the invertebrate zoology course at the Marine Biological Laboratory, has been appointed graduate assistant in zoology at Vassar College.

M.B.L. TENNIS CLUB

The annual tournaments of the Tennis Club are under way; the finals in some of them are scheduled for this afternoon.

In the mixed doubles, the finals will see S. Lumb and D. Humm playing against L. Te Winkel and D. Lancefield.

In the men's singles, the following results were reached in the second round: Stunkard defeated Mavor, 6-2, 6-3 and Evans defeated Williams, 6-1, 6-1.

In the women's singles, Mary Chamberlin will be one of the finalists, having defeated G. Saunders, 6-2, 4-6, 6-4.

In the men's doubles Stunkard and Evans have qualified as finalists by defeating Jones and Speidel, 6-2, 6-1.

Dr. Kenneth S. Cole, associate professor of physiology at Columbia University, has received a Guggenheim fellowship to study the electrical aspects of the structure and function of the living nerve.

Dr. George L. Kreezer, assistant professor of psychology at Cornell University, has been awarded the Snyder grant of $1,000 to continue his research on the effect of pre-natal deficiences in nutrition upon the development of behavioral capacities in the rat. He worked at the Marine Biological Laboratory in July.

Mr. James McInnis, manager of the Supply Department of the Marine Biological Laboratory, has been appointed chairman of the Falmouth Division of the Massachusetts Committee on Public Safety, which has charge of all home defense activities. His latest assignment is to organize a Gasoline Conservation Committee.

Dr. C. E. Wilde, Jr., instructor in zoology at Dartmouth College, who reported recently for work at the Marine Biological Laboratory, was drafted shortly after his arrival and is now stationed at Camp Edwards.

Dr. Robert Chambers left on Tuesday morning for Cleveland.

The Eleventh Annual Meeting of the American Malacological Union will be held at Thomaston and Rockland, Maine, from August 26 to 29.

A general scientific meeting will be held Tuesday and Wednesday, August 26 and 27 in the Auditorium for the presentation and discussion of work done at the Marine Biological Laboratory during the present season.

At the staff meeting of the Woods Hole Oceanographic Institution on August 11, William C. Derrington of the Fish and Wildlife Service presented a paper on "Fluctuations in Fish Population and the Problem of Optimum Yield."

M.B.L. CLUB

Tomorrow evening the third of the occasional Sunday phonograph record concerts will be presented. The opera "Dido and Aeneas" by Henry Purcell will be played at 7:15 P. M. in the Fireplace Room.

On Monday, because of the lecture in the Auditorium at 8:00 P. M., the concert will begin at 9:00 and will last only forty minutes. The following numbers will be presented: Sibelius, "Romance in C for Strings"; Sibelius, "Swan of Tuonela"; Sibelius, "Symphony No. 7 in C Major."

INVERTEBRATE CLASS NOTES

A well organized and extensive study of the marine annelids together with two long field trips made up the scheduled bill of fare for the past week. In addition to the dissection of *Nereis* and *Arenicola*, we tried identifying the different local species with the help of a key. Through the efforts of the collecting crew fifty-one of the fifty-nine species were available. The large number of people working in the lab Sunday indicates the interest taken in this aspect of the work.

Though most of a week had elapsed since Dr. Rankin's last lecture, a student was recently heard laughing explosively, having just caught on to one of the former's "jokes." The time lag is understandable: "The difference between a bird with two wings and a bird with one is just a matter of opinion." Explanation supplied on request.

Our collecting trips to Kettle Cove and Cuttyhunk proved very successful, both from the point of view of the number of specimens collected and of sheer good fun. For there is the two-hour boat trip to Cuttyhunk, with group singing, jokes, and banter; the sustaining if brief picnic lunch, and the thrill of discovery. As many as 135 specimens were collected by a single team. The old *Winifred* whistled herself hoarse before the last team reluctantly gave up the chase.

Saturday night twenty-four invertebrates left the dance, boarded row boats, and made for Devil's Foot Island. Two trips were required to ferry the entire group. Supper of hamburgers was followed by ever popular group-singing, this to be interrupted by peals of thunder. The wind-driven rain sent the picnicers scurrying to the boats. The trip to the mainland, ordinarily requiring twenty minutes, required considerably more time with overladen boats and opposing wind and tide. Indeed, the last group, in spite of the capable oarsman, was obliged to abandon the crossing. The boys succeeded in dragging the boat to Ram Island, where, in the crowded shelter of the overturned boat, they awaited a more favorable opportunity. After about two hours, the eight adventurers once more headed homeward only to find they lacked one oarlock. After contriving a substitute from the remains of a chair and several shoe-laces, the group arrived at 5:30 A.M., to be welcomed by a hardy few, determined to miss none of the fun.

What with the annelid drawings being due Monday evening at seven, a beach party was organized to take advantage of the unexpected holiday. A marshmallow roast at Gansett beach made for less threat from tide and storm, and about as much fun as Saturday's adventure.

A certain member of our class, now called "the ancient marooner," left his girl stranded at Penzance Point Light when the latter shoved the boat into the Woods Hole current. The valiant collecting crew effected a rescue three hours later.

—*Louise Gross and Bill Batchelor*

PROTEINS IN ACTION

(Continued from page 145)

cal problems, is of course a linear pattern of molecules. It is to be distinguished clearly from an atomic fibril or chain which is a linear pattern of atoms, such as have enjoyed for no good reason so far as I can see, some vogue in certain quarters in diagrams of supposed physiological situations. When the native protein units are arranged in two dimensional formation we shall talk of a fabric or skin, which may be open like the sail of a ship or a piece of paper, or closed like the surface of an orange or of a torus or anchor ring. A set forming a three dimensional array completes the three basic categories. Each type is of course flexible, somewhat extensible, has some tensile strength. Each type represents a system having organization in a definite sense, even if this sense is not entirely easy to make perfectly precise. The specificity we have talked about as characteristic of all protein structure systems is present, present as before explained in a dual form. There is specificity of the R-group patterns on the faces of the native protein units which are disengaged and there is specificity in the interlinks, derived, of course, from the R-group patterns of the faces which are interlinked.

It need hardly be pointed out that combinations of these three basic types will give compound fibrils of any cross section, compound fabrics of any thickness, and so on. Since it is essential to pay special attention to the orders of magnitude involved, it should be noticed that a fibril of length ten microns will contain something of the order of 3000 units the size of insulin and that for a cross section of one tenth of a micron in diameter we shall need some thirty strings of single units arranged in parallel. A skin, one molecule thick, will contain say 5×10^4 units the size of insulin per square micron, so that a protein complement of the order of 10^{-12} mg. per square micron as found in the red cells of many species can correspond to a fabric, one molecule or at most a few molecules thick.

In the case of cytoplasm, structure systems within such a membrane can be devised forming one and the same system with the membrane, having as high a proportion of water to protein as we wish. Thus the structure systems already devised (Cold Spring Harbor Symposium, 1941)

in which long molecular fibrils are joined in (say) four-way native protein units (topologically of the type of the diamond structure) give water complements of 70 to 99 per cent, corresponding to fibrils of lengths say 100 Å to 0.7 microns. Evidently with such structure systems even the notorious jellyfish, with its immense water complement, can be accommodated.

It is not possible to demonstrate in detail on this occasion all the ways in which these simple types of structure system suggest interpretations of many facts regarding cytoplasm and membrane behavior. It seems of importance to call attention to the localization of different interlink types in different parts of the membrane structures, which explains why permeability properties can be modified by fewer molecules than are required to cover the surface with a layer one molecule thick. Permeability to certain polar molecules will evidently, on this model, be profoundly modified in the presence of suitable molecules only at certain interlink regions of specific nature where such molecules normally enter. Size is of importance, we notice, specially with respect to molecules entering the rather definite pores, outlined by polar groups rigidly held in hydrogen bridges. Size will be less important in the case of the penetration by the comparatively non-polar molecules whose natural entry points will be the hydrocarbon interlink regions, in which suitable groups cushion against one another, no definite pores of strictly defined size being present.

Among a great variety of relevant considerations, the fundamental one appears once more to relate to specificity. The bewildering variety of red cell membranes is here seen to be compatible even with a great economy of native proteins. Thus, even if, as some have suggested, there is a considerable similarity in the proteins involved, exploitation of the many ways in which the same units can form structures of utterly different specificities by different patterns of interlinkings will provide sufficient variety to cover all the facts so far elicited. As an example of the way in which specific permeabilities may be dependent upon quite minor and in any case strictly localized R-group situations a possible correlation between permeability to urea and the presence of the carboxylic acid groups in the form of glutamine and aspargine may perhaps be suggested. May it not be possible that reptiles differ from the birds in some such way? As an example of ways in which local specificities of membranes may have a far reaching influence on structure systems, we would call attention to the possibility that the hydrophilic R-group patterns on the outside of a plant plasma membrane may determine the pattern in which cellulose is laid down.

The Protein in Modern Dress

In summing up, I should like to come back to my starting point. The native protein unit in modern dress, the true megamolecule, is essentially a structure-forming substance; as such it fulfils the essential requirement of the biologist. Its capacity to form structures, we have seen, allows us to picture systems of immense size and immense chemical complexity in which great flexibility is conjoined with rigid foci. Nevertheless such systems are built in essence upon simple themes. Specificity of the units implies specificity also of interlinks and it is this higher order of specificity which, I would suggest, is the true explanation of organization which has been postulated by biologists for so long. "We must," said Brücke writing in 1861, "attribute to living cells, beyond the molecular structure of the organic components which they contain, still another structure of a different type of complication. It is this which we call by the name of organization." Again in the writings of Wilson we read, "We assume as our fundamental working hypothesis that the specificity of each kind of cell depends essentially upon what we call its organization. . . . We cannot, it is true, say precisely what organization is, but we can hardly think of it as other than some kind of material configuration of the protein substance and one that involves a differentiation of parts and their integration to form a whole, as Herbert Spencer long since urged." Into all these statements we can, without forcing, read the newest picture of the native protein, which is thus seen to be the direct descendent and the present-day embodiment of Pflüger's living molecule, postulated in 1875 and Verworn's biogen. Perhaps the clearest exposition of this point of view is the statement which occurs in the course of Whitman's celebrated attack on the cell theory delivered as an Evening Lecture at this Laboratory in 1893 and published in the first volume of "Biological Lectures." Leaving the accepted cell theory in rags and tatters behind him, the first Director of the Laboratory proceeds to discuss what can be the nature of the "formative processes" in living matter. "The answer to our question," he says, "may be difficult to find, but we may be quite certain that when found it will recognize the regenerative and formative power as one and the same thing throughout the organic world. It will find . . . a common basis for every grade of organization and it will abolish those fictitious distinctions we are accustomed to make between the formative processes of the unicellular and multicellular organisms. It will find the secret of organization, growth, development, not in cell-formation, but in those ultimate elements of living matter, for which idiosomes seems to me an appropriate name. . . What these idiosomes are, and

how they determine organization, form and differentiation, is the problem of problems on which we must wait for more light. All growth, assimilation, reproduction and regeneration may be supposed to have their seat in these fundamental elements. They make up all living matter, are the bearers of heredity and the real builders of the organism."

Logic and History

In this statement, attributing to the protein not only a dominant but the preeminent role in biological structures, Whitman perhaps goes further than many, aware of the great importance of lipids, carbohydrates and other molecules, would be willing to go today. But especially in view of the protein crisis at the present time, I for one would wish to give great weight to the considered judgments of practicing biologists, who in their wide experimental studies may perhaps get from the feel of the material a truer view, even if it be partly intuitive, than those who lack such intimate contacts with protein in its living state. After all, it is (unhappily) only the biologist who has a special stake in the attack on the living protein: alone aware of all the challenges that a living organism can make, he must decide on the adequacy of theories of protein structure in the last resort.

To my way of thinking, the idea in the mind of Whitman and many others such as Fletcher who preceded him, when translated into modern dress, finds a ready interpretation in the picture of the native protein drawn from the work of Svedberg, the work of the immunologists, the work of the protein crystallographers, which finds also generalized and precise expression and confirmation in modern and classical geometry. The native protein, the megamolecule, the most specific of all substances, is a structure-forming unit whose stability indeed depends upon its state of association. Such stabilization is accomplished, apparently, in the mixed structure systems in which protein, water and other substances are organized with superspecificity. But the essence of all such structure systems resides, so far as I can see, in one thing, namely the maintenance intact of the native protein structure. In saying this, I am, of course, only saying what all workers with living material have always known, at least in their unconscious minds. What, then, is this essential structure, upon which, if these workers have read the facts aright, life itself depends? I suggest it is the closed fabric structure, a distinctive structural type (as Wu has shown is absolutely necessary) which is potentially immortal as it passes from generation to generation through the ages and yet is of the utmost lability and can break down in myriad ways. The general tenor of one's thinking, perhaps also of one's physiological processes, will no doubt play a large part in determining for each of us whether the idea of molecular structures in the form of closed atomic fabrics, carrying substituents rooted in spatial patterns on the surface, appears fantastic and obscure, as it has been adjudged by some, or eminently reasonable and straightforward, as it has been adjudged by others. Even were the majority reaction of the former type, we need hardly be alarmed. Such matters have their relevance in the history of Science, but none in the logic of Science. It has happened more than once that an idea, ultimately useful as a tool in subduing the seeming disorder of the external world, did not at first sight appear sober and reasonable to *all* the most distinguished contemporary thinkers. To establish its right to a place in the sun, an idea requires no universal assent. To be useful, it is sufficient that some should foster its development by attempting to understand it and that others should study its implications by experiment and observation and reflexion. Now that its century-long period of gestation is over and its embryonic stages are nearing completion, we await with confidence not unmixed with impatience the serious study of its implications in biological structure problems.

(This article is based upon a lecture delivered at the Marine Biological Laboratory on July 25.)

INTERPRETATIONS OF EFFECTS OF CO AND CN ON OXIDATIONS IN CELLS

(Continued from page 137)

different effects upon the rate of O_2 consumption were produced, depending upon the stage of development. The results were as follows:

Methylene blue produced a rate of 130% in the unfertilized stage of *Arbacia* as compared with the control; 180% in the first cleavage stage; a slight increase in the pluteus and either a decrease or no effect in the gastrula stage.

Carbon monoxide produced a slight decrease to about 92% of the controls in unfertilized eggs; a considerable decrease to about 20% of the normal in the first cleavage stages, after which the decrease was less, so that in the late gastrula stage, the rate was 50%. In all of these cases the addition of methylene blue increased the rate about 10%.

KCN caused a varying degree of decrease. For example in *Asterias* eggs, unfertilized, the rate was 63% of the control; while in the blastula stage of *Arbacia* it was 25% and in the late gastrula stage of *Arbacia* it was 6%.

These experiments suggest new aspects of the respiratory enzymes and the redox systems direct-

ly associated with them. In living cells methylene blue is about half reduced and half oxidized (the ratio of the sum of the reductants over the sum of the oxidants,

$$\frac{[\text{Red}]}{[\text{Ox}]} = \frac{50}{50}),$$

so that its maximum poising action is available. Any system has its maximum electron transference as the potential changes when the ratio of oxidants to reductants is one. Since methylene blue induces different changes in the rate of O_2 consumption at different stages of development of the embryo, one may well inquire whether the optimum redox potential at which the respiratory enzyme and its associates function is different in different stages of the same animal. If the redox potentials are different, then the enzyme systems must be different or they must assume different roles at different stages of development.

The above interpretation would also explain the differences in cyanide-sensitivity as found in different types of living cells. Since cyanide is known to produce a negative redox potential, those respiratory systems which normally have a 50/50 ratio at the redox potential approximating that produced by cyanide, would be less affected than systems whose potentials are farther removed. The former would be known as "cyanide-insensitive" systems and the latter as "cyanide-sensitive". In other words, if the ratio of the sum of the oxidants over the sum of the reductants was changed from 50/50 to 5/95 by KCN, there would be present a greater concentration of reductants which could not reverse back and forth between oxidized and reduced forms. This would produce a slower rate. Furthermore, if the redox potential of the systems were not changed by cyanide, then no change in the rate would occur. This theory eliminates the necessity of trying to explain the action of cyanide by assuming that there is an affinity with cyanide in one case and none in other cases. This theory amplifies a former hypothesis of the writer on the action of cyanide in living cells, namely, that there is no combination with the respiratory enzyme, but merely a lowered redox potential, "freezing" in the bivalent form a large concentration of electrons of the Fe of the heme radical which would otherwise be able to shift reversibly back and forth from the bivalent to the trivalent form. This shift, $Fe^{++} \rightleftharpoons Fe^{+++}$, which must occur for respiration, remains mainly in the Fe^{++} state, so that the concomitant transfer of activated hydrogen to oxygen, which is the object of respiration, cannot take place.

In the case of methylene blue, the same principles may be applied. If methylene blue raises the redox potential, for example, of a component of a respiratory system so that its ratio of oxidants to reductants is 50/50, the rate of respiration should be increased. If however the system affected has its equilibrium ratios changed by the addition of methylene blue so that the concentration of reductants is greater than that of oxidants, or vice versa, then there should be a decrease in the rate.

This tentative explanation is offered of the effects of these accelerators and inhibitors on O_2 consumption. It is difficult to account for the effect of CO, because so far, no stable potentials have been found in the presence of CO with the methods in use.

Theorell's (*Publications A.A.A.S.* №14, 1940, p. 136) conclusions on cytochrome C which also has a heme radical as its active group and a high redox potential, are of interest in this connection. He states that at physiological pH values, i.e. around neutrality, there are amino residues attached to the Fe of the heme radical which prevent it from forming additional compounds with cyanide or CO. Only in alkaline solutions does ferricytochrome-C form a complex with cyanide; and only in acid and alkaline solutions does ferrocytochrome-C form complexes with CO. These explanations may well apply to the respiratory enzyme although nothing so definite is at present known. The experiments of Clark (*Cold Spring Harbor Monographs. VII,* 18, 1939) and those of Barron (*Jour. Biol. Chem. 121,* 285, 1937) on hemochromogens and cyanide were done at high pH values up to pH 13. Their results may well fit in with the above theory of Theorell when alkaline solutions are used, but should not be used to explain what takes place at pH 7.0 in living cells.

II. *Effects on Cleavage.*

A few of the observations on cleavage are as follows: KCN produced multiple aster formation without cell division even in the presence of methylene blue at this concentration. The blastula stage of starfish remained alive 8 days without further development. CO produced cytolysis but methylene blue protected the embryo or egg from cytolysis.

Development of the embryo took place after the removal of CO. However development in the blastula stage of *Arbacia* was stopped even when CO was replaced by air. Other stages were in the main not permanently affected by CO. Methylene blue has doubled the life of both *Arbacia* and *Asterias*; it produced faster development, it increased the size of the plutei in *Arbacia* from 280 μ, an average size in the controls, to 420 μ in length.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 5.)

THE COLLECTING NET

Vol. XVI, No. 9 SATURDAY, AUGUST 23, 1941 Annual Subscription, $2.00 Single Copies, 30 Cents.

THE UTILIZATION OF AMMONIA BY *CHILOMONAS PARAMECIUM*

DR. JOHN O. HUTCHENS
The Johns Hopkins University

Chilomonas paramecium is a cryptomonad flagellate about 30 micra long and 15 micra wide which was found by Mast and Pace (*Protoplasma*, Bd. 29, S. 326-359, 1933) and subsequently by others to grow in a variety of solutions using single amino acids, urea or ammonia as a source of nitrogen, and lactate, acetate, formate, or CO_2 as a source of carbon. Table I (Solution A) shows the composition of one of the solutions used by Mast and Pace and used by me in earlier work (Hutchens, *J. Cell. and Comp. Physiol.*, V. 17, pp. 321-332, 1941). It will be seen that ammonia is the sole source of nitrogen, and that acetate is the only source of carbon. In such solutions the chilomonads attain a population of only 5-6 thousand/cc. and die on the fourth and fifth days of the culture. They can be subcultured indefinitely, however, if transfers are made on the second or third days.

(*Continued on page* 170)

M. B. L. Calendar

TUESDAY, August 26, 9:00 A. M.
General Scientific Meeting
Continued at 2:00 P. M.

WEDNESDAY, Aug. 28, 9:00 A.M.
General Scientific Meeting

WEDNESDAY, Aug. 28, 8:00 P.M.
Motion pictures of local fauna and flora, George G. Lower.

THURSDAY, August 29, 8:00 P.M.
Lecture: Dr. Milton Levy: "Chemical Studies of the Chick Embryo."

FRIDAY, August 30, 8:00 P. M.
Lecture: Dr. Rudolf Schoenheimer: "The Dynamic State of the Body Constituents."

THE OFFICIAL MEETINGS OF THE MARINE BIOLOGICAL LABORATORY

DR. CHARLES PACKARD
Director

The meetings of the Corporation and Board of Trustees of the Marine Biological Laboratory, held on August 12, were uneventful in the sense that no controversial matters came up for discussion. The Officers and Trustees eligible for re-election were voted in by unanimous consent, as were also the three new Trustees and the fourteen new members of the Corporation. Dr. W. J. V. Osterhout was elected a Trustee Emeritus. The Treasurer, Mr. Riggs, reported that the Laboratory is free of all indebtedness and that its finances are in good condition. The number of investigators and students in attendance this summer is only slightly below the average of the past five years, which is 485, though considerably below that of 1937 and 1940. In those seasons, more than 500 were registered, and the Laboratory was uncomfortably crowded. We may congratulate ourselves that in spite of wars and rumors of wars, investigation

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THE MARINE BIOLOGICAL LABORATORIES OF WOODS HOLE

and instruction have gone on almost unhampered.

The completion of the new wing of the Library marks another stage in the development of this important part of our institution. In earlier days it was housed on the Protozoology Laboratory; then in Room 217 of the Crane Building, which soon proved inadequate. When the Brick Building was erected, the shelf space allotted to the Library was deemed sufficient to accommodate the growth of many years. But the rapid increase in the number of back sets, current periodicals, and separates, made imperative a very substantial addition to the stack room. This we now have, thanks to the generosity of the Rockefeller Foundation. The excellent arrangement of the journals and the adequate provision for readers make the Library a most attractive place to the investigator.

During the past year, the storage battery, which was damaged by the flood of 1938, was dismantled. This means that we now have no source of light and power other than the town supply. If this fails, as it has on more than one occasion during the present season, our pump stops, and our supply of running sea water is rapidly depleted. Now sea water is our life-blood; without it we could not continue our observations and experiments for a single day. It is necessary to keep the storage tank full so that if the pump stops temporarily there may be enough water to supply our needs until the electric current is restored. Only by using sea water sparingly can this be done. Investigators can ensure the continuance of their experiments by using a minimum of sea water.

The Supply Department has made two changes in the method of collecting and keeping certain kinds of live material. It no longer uses its own fish trap, but relies on the catch taken in the much larger nets of local fishermen. This plan has worked out very well this season. Urchins and stars, dredged from the sea bottom, are either delivered to investigators directly from the boat, or placed in shallow tanks indoors, where they apparently keep in better condition than they did in the wire cages under the floats.

Dr. Pond called attention to the fact that many items essential to experimental work are becoming increasingly difficult to secure. For example, copper, brass, tin, rubber and aluminum can be obtained only in odd lots, if at all. Acetic, citric and carbolic acid, solvents and many other chemicals are in the same class. Fortunately ethyl alcohol can be purchased, though some restrictions are placed on it. Every effort will be made to obtain these supplies, but obviously investigators can be furnished only very limited amounts.

The relation of the Laboratory to the present emergency was summarized by Dr. Lillie in the following words:

"During the present international and national crisis it is both the privilege and duty of educational and research institutions to fulfill their normal functions as effectively as possible. We have done so this season and will continue so to act. It is not necessary for me to emphasize to the members of the Marine Biological Laboratory the value and dignity of our role in the advancement of science and education, nor, I am sure, the responsibility that rests upon us to defend and preserve our institution.

"We shall, of course, stand ready to make sacrifices in the interests of national defense if called upon to do so. Our location might make the use of some of our facilities for inshore patrol work valuable to the Navy Department; I understand that there are plenty of rumors about, that they may be requisitioned in whole or in part. We have had no official notification that this is likely to be the case, and we feel assured that our own interests will be respected as far as possible in any demand that might be made. It is my own opinion that it is improbable that any such demand will be made this year, but I have no opinion about the subject beyond that time."

COMPARISON OF THE RESPIRATORY RATES OF DIFFERENT REGIONS OF THE CHICK BLASTODERM DURING EARLY STAGES OF DEVELOPMENT

Dr. Frederick S. Philips

Osborn Zoological Laboratory, Yale University; Department of Physiological Chemistry, Yale University, for the academic year 1941-1942

The consideration of the regional differences in embryonic processes which exist in the head-process embryo of the chick, or for that matter in any embryo, demands an analysis of the nature of the biochemical phenomena which must be involved in such morphogenetic activity. A few investigators have already attempted the beginnings of this sort of analysis in the chick. Rulon, 1935, and Miller, 1941, have both found that the vital dye, Janus green, under anaerobic conditions is

The Collecting Net was entered as second-class matter July 11, 1935, at the Post Office at Woods Hole, Mass., under the Act of March 3, 1879, and was re-entered on July 23, 1938. It is devoted to the scientific work at marine biological laboratories. It is published weekly for ten weeks between July 1 and September 15 from Woods Hole, and is printed at The Darwin Press, New Bedford, Mass. Its editorial offices are situated in Woods Hole, Mass. Single copies, 30c by mail; subscription, $2.00.

most rapidly reduced in the region of the embryo which contains Hensen's node. Jacobson, 1938, has reported a diminution of glycogen and an increase in lipoid material in epiblast cells which are invaginating in the primitive streak. The present work is a study of the rate of oxygen consumption in different regions of the head-process embryo in order to investigate the existence of possible correlations between one phase of respiratory metabolism and the various regional differentiations in embryonic activity previously described.

A second point of interest in the present work arises out of the recently published observation (Philips, 1941) that there is an increase in the rate of oxygen consumption of the whole chick blastoderm during the first four hours of incubation. In extension of this earlier observation the present investigation considers the question of whether a respiratory enhancement takes place in the area pellucida of the blastoderm during this same early period. It also considers the changes in rate of oxygen consumption of the embryonic material up to the time of the formation of the head-process embryo.

After a preliminary period of incubation at 38°C. blastoderms were removed from the yolks of eggs (White Leghorns and in a few cases, White Rocks) in Ringer-buffer solution. Dissections of the embryonic regions were carried out with fine glass and steel needles and the area of the isolated pieces was measured by means of an ocular micrometer. Oxygen consumption was determined in the Cartesian diver microrespirometer of Linderstrøm-Lang with the modifications introduced by Needham, Boell, and coworkers. The volume of the divers used was about 30 c. mm. with rate constants of about 20 m. μl. O_2 consumed for every centimeter change of the levels of the Brodie fluid. The embryonic tissues after isolation from the embryo were placed in the diver bulbs with 2 c. mm. of Ringer-buffer containing 0.4 per cent glucose. A stream of oxygen was passed through the divers before placing the alkali seals in their necks. After the oxygen consumption had been followed for about two hours, the pieces were removed from the divers and their total-nitrogen determined. The nitrogen estimation was made by a modification of the micro-Kjeldahl method of Needham and Boell, 1939. Dr. Boell, who plans to publish a description of this modification in the near future, has found it reliable between 0.5 and 25 γ N. Since the value of the total-nitrogen was known, the Q_{O_2}' of the embryonic pieces could be calculated as the m. μl. O_2 consumed/hour/γ N.

In order to test the reliability of the described techniques for the present analysis the rate of oxygen consumption was compared in right and left halves of the area pellucida of the same head-process blastoderm. The halves were produced by a median cut through the head-process and primitive streak of the isolated area pellucida. Each half contained about 2 γ total-nitrogen and consumed about 0.25 c. mm. O_2 in two hours. The ratio of the rate of the right half to that of the left half was 1.02 according to the average of results from twenty determinations. Moreover, the average Q_{O_2}' of 24 right halves and 28 left halves was in both instances 78. Since there is such a close agreement in the value for the two halves, it appears that the techniques used for the present determinations give reliable results. It may also be concluded that the rate of oxygen consumption under the conditions of the present experiments is similar in both halves of the area pellucida of the head-process embryo.

The next observations concern the study of the different regions of the head-process pellucida area. The head-process embryos used varied in development from the early head-process to the early head-fold stages. The area pellucida was divided into six pieces containing respectively the head-process, node and anterior streak, middle streak, posterior streak, and right and left lateral regions. In order to obtain sufficient material for the nitrogen analyses the same regions from two or three blastoderms were analysed together in the same divers and Kjeldahl flasks. Two or three pieces of the node and anterior streak region contained about 2 γ N.

In Table I it can be seen that the head-process, node and anterior streak, and middle streak regions have very similar Q_{O_2}' values. The other regions have somewhat higher rates, but from the values of the standard deviations it does not appear that the differences are significant. In a few determinations of the latter regions the amounts of material were small enough to give serious errors in the micro-Kjeldahl determination used. It is also probable that in these determinations the total nitrogen values were incorrect due to losses of tissue material during the transferring procedures which would result in rate values that were too high. However if the small differences noted are real, they may be of considerable importance in the regional differentiation of the head-process embryo.

The results of the observations on the Q_{O_2}' of the area pellucida from the stage of the unincubated blastoderm to the 37-hour embryo (11-12 somites) show that there is an increase in the rate during the first 17 hours of development and then the rate remains similar through the latest stage determined. For these determinations as much of the area pellucida was used as possible. The Q_{O_2}' of the tissue in the unincubated blastoderm was about 33. By the definitive streak stage the value had increased to about 75. Similarly the values for the head-process, 5-6 somite, and 11-12 somite

Table I.

The Q_{O_2}' (m. μ 1. O_2 consumed/hour/γ N.) of different regions of the area pellucida during the first hour of measurement. Averages of 10 determinations are given with the standard deviations.

Head-Process	Node and Anterior Streak	Middle Streak	Posterior Streak	Right Lateral	Left Lateral
77 ± 9.5	75 ± 7.3	74 ± 10.8	85 ± 13.5	89 ± 19.8	92 ± 19.9

stages were respectively 81, 75, and 78. These latter values may be converted into dry weight Q_{O_2} rates by dividing them by the factor 9. (This latter factor is based on the observation that the dry weight of chick embryos older than 4-days contains about 11 per cent total-nitrogen.) It then can be seen that the present observations give values for the stages between 17 hours and 37 hours of development which fall between 8.3 and 9.0 c. mm. O_2 consumed/hour/mg. dry weight. Using the Fenn respirometer similar values have been found for stages ranging from the 1-day area pellucida to the 3-day embryo, namely 9.2 to 12.1 (Philips, 1941). With the use of the Warburg apparatus Romanoff has shown in unpublished experiments that from the 4-day embryo to the 7-day embryo the Q_{O_2} also remains at a similar level, between 9.0 and 10.5. These data indicate that the level of the rate of oxygen consumption of the whole area pellucida and the embryo remains relatively constant between the definitive streak stage and the seventh day of development.

The Q_{O_2}' of the area pellucida of the unincubated blastoderm is of the same magnitude as the value for the whole blastoderm at this stage (Philips, 1941). Similarly the increase in rate of the area pellucida during the first four hours of incubation is like that found in the whole blastoderm. The continued increases in rate of the area pellucida up to the seventeenth hour of development have a possible explanation. There appears to be very little change in the total nitrogeneous material contained in the whole area pellucida during this early period of development when the cell-number is being tremendously increased. Thus this region contains about 3 γ N in the unincubated blastoderm and about 3.9 γ N in the head-process embryo. Accompanying the increasing cell-number there is, however, a marked increase in the total amount of oxygen consumed/hour, from about 100 m. μ1. in the pellucida area of the unincubated blastoderm to about 313 m. μ1. in the head-process stage. It, therefore, appears that during the first 17 hours of development nitrogenous, as well as other materials, are being converted from inert, storage elements in the form of yolk granules into actively metabolizing cellular constituents. In subsequent stages of development the increase in the total materials of the area pellucida and embryo more or less keeps pace with the increasing cell-number and oxygen consumption.

One of the stated objects of the present work was the investigation of possible correlations between differences in the rate of oxygen consumption of the various regions of the head-process embryo and the differences in embryonic activity known to exist in these regions. Under the specific conditions of the present experiments no regional differences of any significance have been observed with regard to the rate of cellular oxidations. However, caution must necessarily be observed in relating this condition to the various regionally differentiated embryonic activities of the head-process embryo. It is entirely possible that the embryonic activities may be suspended on removal of the regions from the embryo and during the measurement of their respiration *in vitro*. Nevertheless, with this possibility in mind it is apparent that these results do not support Child's axial gradient theory of development. No gradient in the rate of cellular oxidations has been found where according to the theory it ought to exist. Furthermore, on the basis of the theory one might expect that embryonic stages as widely different in embryonic activity as the definitive streak, head-process, and 7-day embryo would possess widely differing rates of oxygen consumption. This is not the case.

There still remains the apparent discrepancy between the results of Rulon, 1935, and Miller, 1941, and the present experiments. The results of these authors may be used as evidence proving the existence of a respiratory gradient in the chick head-process embryo. There is one objection which may be raised to such an interpretation. The node region which shows the highest rate of Janus green reduction under the induced anaerobic conditions of their experiments is the thickest

region of the head-process area pellucida. Such a region ought to show the presence of the reduced form of the dye before other regions since it contains a greater number of cells per unit area of pellucida area material. In confirmation of this morphological picture of the head-process embryo the present observations show that there is the greatest amount of nitrogen per unit area in the node region. Furthermore, the relative thicknesses and the relative quantities of nitrogenous material per unit area agree with the relative rates of dye reduction reported. It seems unlikely that the dye experiments really show a gradient in the respiratory activity of individual cells in the various observed regions.

It cannot be denied that when it is possible to make determinations on even smaller amounts of material than were used in the present work some significant differences in rate of oxygen consumption may be found. Furthermore, oxygen consumption is only one respiratory activity of living cells. When other metabolic properties like glycolysis are investigated for the same stages, regional differences may be discovered.

By way of conclusion this work is considered a phase of descriptive embryology. It might be called specifically chemical morphology. Complete analytical investigations based on the present data can only come sometime in the future when the very general function, the Q_{O_2}, can be dissected into the component factors from which it is derived. Only these factors can give some insight into the way energy from combusted food substances is used in the various embryonic processes which result in differentiation. Furthermore, unlimited possibilities exist in the manifold constitution of all the components of the respiratory systems for the provision of a variety of metabolic differentiations without it being necessary that differentiation be reflected in the over-all rates of oxygen consumption.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 5.)

STUDIES ON CONDITIONS AFFECTING THE SURVIVAL *IN VITRO* OF A MALARIAL PARASITE (*PLASMODIUM LOPHURAE*)

Dr. William Trager
Rockefeller Institute, Princeton, N. J.

The malaria parasites comprise a rather homogeneous group of protozoa which inhabit the blood and blood forming cells of certain vertebrates. They are transmitted from one vertebrate host to another by an insect vector in which they undergo a sexual cycle of development. There are 4 species of malaria parasites of human beings, no one of which has ever been successfully transmitted to any other host. There are several species of malaria parasites of monkeys and these again are highly specific for monkeys. Then there are about 12 species of malaria parasites of birds, which are specific for certain groups of birds. These bird malaria parasites provide good material for experimental studies in malaria and one of them, *Plasmodium lophurae*, has been used for the work to be reported here. It is an especially good organism for experimental purposes as it is infectious to the young chicken, a cheap and readily available host animal.

All the malaria parasites have an essentially similar life cycle. In the vertebrate host, a small form, the merozoite, invades a red blood cell and grows in size. Its nucleus then divides repeatedly to form a segmenter having perhaps 6 to 20 nuclei, depending on the species. A small amount of cytoplasm gathers around each nucleus and the host red cell bursts and liberates the newly formed merozoites. Each merozoite is then capable of infecting a new red cell and repeating the process. This asexual cycle continues freely in any one host until the host succumbs to the infection or develops a resistance. Under laboratory conditions, the asexual stages can be kept going indefinitely by the inoculation of blood from an infected to a fresh host. Since all the experiments presented in this paper were concerned exclusively with the asexual stages, the sexual stages which occur in the mosquito vector need not be considered.

It is evident from this brief review of the biology of malaria parasites that they are highly specialized organisms. So far as present knowledge goes, they are just as much obligate intracellular parasites as are any of the viruses. No one of them has ever been cultured *in vitro*. Indeed, previous attempts at their cultivation have failed not only of their ultimate object, but have also failed to give much information concerning even the simplest conditions which might favor the survival of the parasites *in vitro*.

It therefore seemed desirable to make a comparative study of the effects of various environmental conditions on the length of life of the parasites outside of their living host. In these studies I have attempted to find, not conditions for the preservation of the parasites in a state of suspended animation, as at very low temperatures, but conditions which would favor their survival under circumstances which would promote either some development or rapid death. Hence, all the experiments were performed at temperatures of

40-42°C., about the body temperature of the chicken. Now, how can we judge the survival of malaria parasites? They are small and typically non-motile. Something can be said as to their condition on the basis of their microscopic appearance in fresh and stained films, but the only reliable criterion of survival is their ability to infect a susceptible host—in this case a baby chick.

The experiments were conducted in the following manner. The media to be tested were placed, with aseptic precautions, in appropriate sterile containers. Blood was taken aseptically from the heart of an infected chicken and the red cells were centrifuged down and resuspended in a special balanced salt solution. Suitable amounts of the parasitized red cell suspension were measured into the experimental containers. These were held in an incubator and removed daily for the taking of a small sample. Each sample was used for the preparation of a stained smear and for the inoculation of two 2-day old chicks. If these chicks showed an infection by the 7th day after inoculation, the infectivity of the sample was designated ++. If they showed no infection on the 7th day, but did show an infection on the 11th day, the infectivity was designated +. If they showed no infection by the 11th day, the infectivity was —.

The balanced salt-glucose solution, called solution K, used for the preparation of media and parasitized red cell suspensions, was prepared on the basis of available knowledge of the inorganic composition of red blood cells, and of certain general considerations. It differs from solutions such as Locke's and Tyrode's chiefly in having a much higher potassium content, a somewhat higher phosphate and magnesium content and a lower pH (7.2). Comparative tests of survival of *P. lophurae* in solution K and in Locke's or Tyrode's showed always longer survival in solution K. Another factor which was early found to have a favorable effect on survival was the presence of red cell extract. This was prepared by freezing and thawing chicken red blood cells once. The frozen-thawed material was suspended in solution K and a clear extract obtained by centrifuging out the nuclei and cell remnants. Such red cell extracts were used in tests of all the other factors to be considered.

Adequate aeration had a marked effect on survival. For example, parasites in red cell extract in a tube to a depth of 15 mm. showed no infectivity by the 3rd day, while those in the same red cell extract in a flask to a depth of 4 mm. still had a ++ infectivity on the third day. In a preparation held in a vial with air bubbled through the infectivity was + on the 5th day, while in the control without a current of air the infectivity was already — on the 3rd day. But if pure oxygen was bubbled through, survival was shorter than with CO_2 — free air.

If fairly dilute red cell extracts were used, an effect of the added carbohydrate in the solution K could be found. Twelve millimols of glucose per liter gave a ++ infectivity on the 4th day, as compared with a — infectivity for 8 millimols of glucose per liter. Again, on the 3rd day, 12 millimols of glucose per liter gave a + infectivity, no added glucose a — infectivity, and 24 millimols per liter a — infectivity, showing a toxic effect of high glucose concentration. Glucose could be replaced by glycogen. Thus, on the 4th day, the infectivity with no added carbohydrate was —, with 0.2% glycogen it was ++. In such dilute red cell extracts added glutathione affected survival. With 12 millimols of glucose as added carbohydrate, the infectivity was + on the 4th day and — on the 5th day in the absence of added glutathione, while in the presence of added glutathione it was ++ on the 4th day and still ++ on the 5th day.

In a similar way, it has been found that the survival *in vitro* of *P. lophurae*, as judged by infectivity, is favored not only by a balanced salt solution of high potassium content, by red cell extract, by adequate aeration, by appropriate concentrations of glucose or glycogen and by glutathione, but also by daily renewal of the medium, by a suitable density of parasites per cu. mm. and by certain concentrations of chick embryo extract, chicken liver extract and chicken serum or plasma.

In the best preparations, at least 40% of the parasites were alive on the 3rd day, at least 20% on the 4th day, about 1% on the 5th day and about 0.05% on the 6th day, the last day of the tests. In such preparations there was a small increase in parasite number on the first day of life *in vitro*.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 12.)

THE EFFECT OF DYES ON RESPONSE TO LIGHT IN *PERANEMA TRICHOPHORUM*

Dr. Charles C. Hassett
Department of Zoology, The Johns Hopkins University

Peranema trichophorum is a colorless flagellate without an eyespot. It moves by swimming or by crawling; when it crawls it moves slowly and is easily kept under observation. It responds to a sudden increase in luminous intensity by ceasing forward motion, bending sharply and then moving off at an angle to its former line of progression. This response is known as a shock-reaction. Mast and Hawk (1936) and Shettles (1937) studied various phases of this response. Shettles

showed that cocaine chloride increases the reaction-time and that strychnine sulfate decreases it. In the present work it was decided to study the effects of dyes on the response.

Raab, in 1900, found that the dye acridine possessed the power to sensitize paramecia to light and that animals in acridine solutions were unharmed if kept in the dark, but were killed if illuminated; the stronger the light, the more quickly they were killed.

The object of these experiments was to ascertain (1) the nature of the effect of dyes on the reactions of *Peranema* and (2) the importance of some of the factors involved, e.g., fluorescence, wave-length absorbed by the dye, structure of the dye molecule.

Neutral red, eosin, rose bengal, orange G, auramine O, brilliant green, methylene blue and Nile blue sulfate were used; each dye was dissolved in Chalkley solution (the culture fluid used in growing the peranemae), and its absorption spectrum was measured on a Coleman spectrophotometer. All of the dyes except neutral red and brilliant green were found to absorb light in relatively limited parts of the visible spectrum; as a group, they cover all parts of the spectrum.

The peranemae were mounted on slides in various concentrations of each dye, then were put on the stage of a microscope, brought to focus in weak red light and suddenly stimulated by the application of a light of 20.35 meter candles intensity. The reaction-time was measured with a stop watch. This was repeated with 50 animals at each of a number of concentrations of each dye. Two hundred animals from the same cultures were used as controls, they were tested while mounted on slides in the regular culture fluid.

The results were as follows: the reaction-time of animals which were not treated with dyes was found to be 12.1 seconds. Several dyes were found to decrease the reaction-time to approximately 1 sec. when the concentration of dye was 2×10^{-4} M, these were rose bengal, eosin, neutral red and methylene blue. More dilute solutions gave longer reaction-times up to 12 sec. for concentrations of 1×10^{-7} M. Nile blue sulfate and auramine O were less effective, relatively strong solutions only produced a minimum reaction-time of ca. 5 sec. Orange G had no effect. Brilliant green increased the reaction-time at a concentration of 5×10^{-5} M; more dilute solutions produced shorter reaction-times down to 12 sec. at 1×10^{-7} M. This dye is much more toxic than the others used and the increase in reaction-time which was observed may have been caused by the toxicity of the dye.

All the dyes used are lethal to *Peranema* even in the dark when strong solutions are used. The observations were therefore made with solutions which had no harmful effects after as long as 24 hours in the dark.

From these results several conclusions can be drawn. Concerning fluorescence, in this experiment the dyes used were of varying degrees of fluorescence, the order being approximately: eosin > rose bengal, neutral red > Nile blue sulfate, methylene blue > auramine O, orange G, brilliant green. Comparing this with the order of effectiveness in decreasing the reaction-time of *Peranema*, it can readily be seen that there is little correlation. These results agree with those of Raab (1900), who found that fluoresced radiation from dyes not in contact with paramecia had no harmful effects.

The molecular structure of the dyes is diverse, (Conn, 1940). Of the four most effective dyes, eosin and rose bengal are similar to each other but differ greatly from neutral red and methylene blue. Hence no specific structural characteristics seem to be involved in photodynamic action.

The wave-length of light necessary to produce photodynamic action is dependent on the absorption of the dye being used. Although white light was used in this work, it has been shown (Blum, 1941), that action spectra coincide closely with absorption spectra. The dyes which were effective in reducing the reaction-time of *Peranema* vary from auramine O, which absorbs light mainly in the region 3900-4300 A to methylene blue, which absorbs mostly red light of 5800-6400 A.

In summary: (1) several dyes were found to exert a photodynamic effect by reducing the reaction-time of *Peranema*; (2) the effect of these dyes is inversely proportional to their concentration; (3) no direct correlation was found between the effect of the dyes and degree of their fluorescence; (4) several types of dye molecules are effective; (5) the active dyes possess diverse absorption spectra.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 12.)

PROGRAM OF THE SUMMER MEETINGS OF THE GENETICS SOCIETY OF AMERICA AT COLD SPRING HARBOR, LONG ISLAND, N. Y., AUGUST 27 TO 29, 1941

Officers of the Genetics Society of America

President, Th. Dobzhansky, Columbia University.

Vice-President, E. W. Lindstrom, Iowa State College.

Secretary and Treasurer, B. P. Kaufman, Carnegie Institute of Washington.

Wednesday, August 27, 8:30 P. M., Blackford Hall, Evening Lecture

A. H. Sturtevant, California Institute of Technology. Comparative Genetics of the Species of *Drosophila*.

Thursday, August 28, 9:30 to 12:45, Davenport Laboratory, Demonstrations

(1) ATWOOD, SANFORD, S., U. S. Regional Pasture Research Laboratory, State College, Pa.: The multiple oppositional alleles causing cross-incompatibility in *Trifolium repens.*

(2) BISHOP, D. W., University of Pennsylvania, Philadelphia, Pa.: Cytological demonstrations of chromosome breaks soon after X-radiation.

(3) BREHME, KATHERINE S., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: A survey of the Malpighian tube color of the eye color mutants of *Drosophila melanogaster.*

(4) BUSHNELL, RALPH J., The University of Connecticut, Storrs, Conn.: Incompatible matings in inbred families of the bean weevil.

(5) DEMEREC, M., HOLLAENDER, ALEXANDER, HOULAHAN, M. B., and BISHOP, M., Carnegie Institution of Washington, Cold Spring Harbor, N. Y., and National Institute of Health, Bethesda, Md.: Effect of monochromatic ultraviolet radiation on *Drosophila melanogaster.*

(6) EIGSTI, O. J., University of Oklahoma, Norman, Okla.: A comparative study of the effects of sulfanilamide and colchicine upon mitosis of the generative cell in the pollen tube of *Tradescantia occidentalis* (Britton) Smyth.

(7) FANKHAUSER, GERHARD, and CROTTA, RITA, Princeton University, Princeton, N. J.: The frequency of spontaneous aberrations of chromosome number among larvae of the newt, *Triturus viridescens.*

(8) GORDON, MYRON, New York Aquarium, New York, N. Y.: Dominant and recessive responses of the Sd factor in natural and domesticated fish populations.

(9) HINTON, O. TAYLOR, and ATWOOD, K. C., Columbia University, New York, N. Y.: A comparison of the specificities of terminal adhesions of salivary gland chromosomes in two strains of *Drosophila.*

(10) KAMENOFF, RALPH J., City College, New York, N. Y.: A cytological study of the embryonic livers (16-18 days) of normal and flexed-tailed (anemie) mice.

(11) KIMBALL, R. F., Johns Hopkins University, Baltimore, Md.: A gene affecting the manner of swimming in the ciliate protozoan, *Euplotes patella.*

(12) LAANES, T., and MACDOWELL, E. C., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: Screw-tail, a new mouse mutation.

(13) POWER, MAXWELL E., Yale Universitty, New Haven, Conn.: Neurological effects of mutants reducing facet number in the eyes of *Drosophila melanogaster.*

(14) RIDDLE, OSCAR, DUNHAM, H. H., and SCHOOLEY, J. P., Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: Genetic hermaphroditism in a strain of pigeons.

(15) ROBERTSON, G. G., Yale University, New Haven, Conn.: Increased viability of homozygous yellow mouse embryos in new uterine environments.

(16) SONNENBLICK, B. P., Queens College, Flushing, N. Y.: The question of cell constancy in various embryonic and larval tissues of *Drosophila melanogaster.*

(17) SPARROW, A. H., McGill University, Montreal, Canada: Spiralization in microspore chromosomes of *Trillium.*

(18) SPENCER, W. P., College of Wooster, Wooster, Ohio: Inherited variations in wild populations of *Claytonia virginica.*

(19) WILSON, G. B., and BOOTHROYD, E. R., McGill University, Montreal, Canada: Differential reactivity in the chromosomes of *Trillium* species.

(20) WILSON, G. B., and SPARROW, A. H., McGill University, Montreal, Canada: Partial fusion of untreated root tip chromosomes of *Trillium erectum* L.

Afternoon Session, beginning at 2 o'clock

INSPECTION OF EXHIBITS prepared by resident investigators of the Department of Genetics, Carnegie Institution of Washington and of the Biological Laboratory of the Long Island Biological Association.

Late Afternoon and Evening

SWIM, PICNIC SUPPER, AND DANCE.

Friday, August 29, at 9:30 A. M., Auditorium of Blackford Hall

(1) BERNSTEIN, MARIANNE E., Carnegie Institution of Washington, Genetics Record Office, Cold Spring Harbor, N. Y.: The incidence and Mendelian transmission of mid-digital hair in man.

(2) LINDEGREN, CARL C., and LINDEGREN, GERTRUDE, University of Southern California, Los Angeles, Calif.: X-ray and ultraviolet induced mutations in *Neurospora.*

(3) BRINK, R. A., and COOPER, D. C., University of Wisconsin, Madison, Wis.: Somatoplastic sterility as a function of the endosperm genotype.

(4) NEBEL, B. R., WILSON, G. B., and MARINELLI, L., New York Agricultural Experiment Station, Geneva, N. Y., McGill University, Montreal, Canada, and Memorial Hospital, New York, N. Y.: X-ray dosage curves in *Tradescantia.*

(5) GILES, N. H., and NEBEL, B. R., Yale University, New Haven, Conn., and New York Agricultural Experiment Station, Geneva, N. Y.: An analysis of the intensity factor in X-ray induced chromosomal aberrations in *Tradescantia.*

(6) CASPARI, ERNST, Lafayette College, Easton, Pa.: Genetic and environmental factors influencing testis color in *Ephest.a kühniella.*

(7) MULLER, B. H., and PONTECORVO, G., Amherst College, Amherst, Mass., and the University of Edinburgh, Edinburgh, Scotland: Recessive genes causing interspecific sterility and other disharmonies between *Drosophila melanogaster* and *simulans.*

(8) LEWIS, E. B., California Institute of Technology, Pasadena, Calif.: The Star and asteroid loci in *Drosophi·a melanogaster.*

(9) STFINBERG, ARTHUR G., McGill University, Montreal, Canada: Further studies on the histological development of the wild type and Bar eyes of *Drosophila melanogaster.*

(10) IVES. P. T., Amherst College, Amherst, Mass.: Allelism and elimination of lethals in American populations of *Drosophila melanogaster.*

(11) NEEL, J. V., Dartmouth College, Hanover, N. H.: A case of high mutation frequency in *Drosophila melanogaster.*

Afternoon Session

INSPECTION OF EXHIBITS — Further opportunity to visit exhibits prepared by resident investigators.

THE GROWTH OF THE LABORATORY

The following table shows the total number of investigators registered at the Marine Biological Laboratory each year since its foundation.

1888	8	1906	68	1924	194
1889	19	1907	60	1925	207
1890	20	1908	52	1926	252
1891	24	1909	66	1927	294
1892	50	1910	62	1928	323
1893	41	1911	82	1929	329
1894	58	1912	93	1930	337
1895	53	1913	122	1931	362
1896	74	1914	129	1932	314
1897	58	1915	137	1933	319
1898	70	1916	129	1934	323
1899	71	1917	129	1935	315
1900	70	1918	93	1936	359
1901	65	1919	134	1937	391
1902	76	1920	136	1938	380
1903	76	1921	172	1939	352
1904	51	1922	182	1940	386
1905	68	1923	176		

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris I. Gorokhoff and Judy Woodring.

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SUMMER ACTIVITIES OF UNIVERSITY OF CHICAGO BIOLOGISTS

Dr. Carl Moore, professor and chairman of the department of zoology, is spending the summer in the north woods at Rapid City, Michigan, working in his own private laboratory on the hormone control of reproduction. In January he received the American Academy of Arts and Sciences award for his work which served as a basis for the isolation and identification of "testosterone", the male sex hormone.

Dr. Sewall Wright, Ernest D. Burton Distinguished Service professor of zoology, is at Cold Spring Harbor, Long Island.

Dr. Alfred Emerson, professor of zoology, is conducting an inspection trip of eastern museums, using his summer home at Hewlett Landing in upper New York as a base. He is a specialist in the field of ecology.

Dr. Frances Oldham, research assistant in the department of pharmacology, is spending her second summer at the Eureka Whaling station at Field's Landing in Northern California. At this station the whales are carved and prepared in public—for an admission charge—before they are marketed. Dr. Oldham will bring back pituitary glands for her work with Dr. Eugene Geiling, professor and chairman of the department of pharmacology.

M.B.L. TENNIS CLUB

Two of the five tennis tournaments this year have already been completed; the finals in the other three are scheduled for this afternoon.

In the finals of the men's singles, D. E. Lancefield defeated A. Stunkard, 6-0, 7-5.

In the finals of mixed doubles, Lancefield and Te Winkel defeated Lumb and Humm, 6-2, 6-2.

The ladies' singles is one of the tournaments scheduled to be completed this afternoon. Mary Chamberlain will be one of the finalists, having defeated P. Saunders, 7-5, 6-3, and she will play against D. Baitsell, who had previously defeated G. Gorokhoff, 6-2, 6-4.

The finals of the men's doubles will see Stunkard and Evans (who have defeated Jones and Speidel, 6-2, 6-1) playing against the winners of the semi-finals in the other bracket—Humm and Hayashi vs. Lancefield and Krahl.

The finals of the junior singles are also scheduled for this afternoon.

WOODS HOLE CHORAL SOCIETY

The fourteenth annual concert of the Woods Hole Choral Society will be presented Monday evening at 8:30 in the Woods Hole Community Hall. Twelve numbers, equally divided between secular and sacred music, have been prepared by the members of the Club, who have been rehearsing twice weekly since the beginning of the summer.

The concert will be conducted by Professor Ivan T. Gorokhoff, director of choral music at Smith College. Dr. Eliot R. Clark is president of the Club, and Dr. Charles Packard is secretary-treasurer. Miss Galina Gorokhoff is accompanist.

The members of the Society, which is composed primarily of Laboratory workers and members of their families, are as follows:

Stella Anderson, Barbara Brainerd, Jane Collins, Grace Crecelius, Emily H. Lower, Edith Mitchell, Louise Thorne Sprenger, Evelyn G. Watterson, Helen Willier, Eleanor Linton Clark, Helen M. Crossley, Eva Stokey Evans, Alma G. Stokey, William J. Blake, Stanley Sprenger, Peter J. Wilhousky, William H. Batchelor, John W. Brainerd, Eliot R. Clark, Boris I. Gorokhoff, Arthur Truslow, and George G. Lower.

The program is as follows:

Break forth, O beauteous, heav'nly light	J. S. Bach
Hail Holy Light!	Alexander Kastalsky
Rejoice in the Lord alway	Henry Purcell
Ave Verum Corpus	William Byrd
Gospodi Pomiluy (Lord, Have Mercy)	Lvovsky
When His loud voice (Jeptha)	Georg F. Handel
Brightly Dawns Our Wedding Day (The Mikado)	Gilbert and Sullivan
Good-day, dear heart	Orlando di Lasso
The Beetle's Wedding	German Folk-Song
A Legend	P. Tschaikowsky
Round the Good Father's Door	A. Arkhangelsky
Moon Magic	Three Russian Folk Songs

M.B.L. CLUB

The ping pong tournament of the M.B.L. Club is well under way with most of the first and some second rounds played off. The tournaments organized this year are men's and women's singles and mixed doubles.

The Monday evening phonograph record concert will not be given this week in order to avoid a conflict with the Choral Club concert.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
August 24	5:30	5:50
August 25	6:15	6:37
August 26	7:01	7:27
August 27	7:49	8:19
August 28	8:41	9:16
August 29	9:35	10:15
August 30	10:35	11:19

ITEMS OF INTEREST

Dr. Dorothy M. Wrinch was married on Wednesday at 12:30 P. M. in the Laboratory to Dr. Otto Glaser by Rev. Ralph H. Long of Falmouth. They are spending their honeymoon on Nantucket Island, and will return later to their house in Quisset. Dr. Wrinch will continue her scientific work as before; she holds a professorship in molecular biology in Dr. Glaser's department of biology at Amherst, and is also professor at Smith College and Mt. Holyoke College. She was given in marriage by Professor John F. Fulton of the Yale University School of Medicine. Dr. Katherine Brehme was her only attendant. The marriage is the first to have been performed in the Laboratory.

Dr. Henry J. Fry died on August 15 in Hartford, Connecticut, after a long illness. Dr. Fry first came to Woods Hole as a student in 1921 and returned as an investigator nearly every year thereafter until 1939. He received his A.B. degree at Muhlenberg College in 1914, and his Ph.D. at Columbia University in 1925. In 1923 he began instructing at New York University, becoming a professor in 1930. From 1933 until his illness he was a visiting investigator at Cornell University Medical College. Dr. Fry was noted for his work in experimental cytology.

New members of the M.B.L. Corporation include: R. Ballentine, M. M. Brooks, Aurin M. Chase, K. W. Cooper, Titus C. Evans, Jean M. Fisher, C. G. Grand, J. Friedrich Gudernatsch, Rudolf T. Kempton, Otto Loewi, F. M. MacNaught, V. Menkin, Isabel M. Morgan and K. G. Stern.

Mr. David D. Perkins, who graduated from the University of Rochester this spring, and who is taking the invertebrate zoology course at the Marine Biological Laboratory, has been appointed a graduate assistant in zoology at Columbia University.

Mr. R. A. Goffin, superintendent of the Bureau of Fisheries, reports that 11,626 visitors to the aquarium registered in the guest book between July 13 and August 16. Since only about one quarter of the visitors register, the actual number probably was about four times as great.

The *Anton Dohrn* left the Oceanographic Institution Wednesday for Nova Scotia to conduct research work in connection with national defense.

At the annual meeting of the Tennis Club, the following persons were elected officers: President, Eric Ball; Vice-President, Margaret Speidel; Secretary-Treasurer, Titus Evans. D. E. Lancefield and E. R. Jones were elected to the executive committee.

Professor A. H. Sturtevant will give the evening lecture at the summer meetings of the Genetics Society of America at Cold Spring Harbor on August 27. He will speak on "Comparative Genetics of the Species of *Drosophila*."

Mr. Robert L. Terry, who was an investigator from the University of Pennsylvania at the Laboratory last year is now a private in the U. S. Army.

Dr. T. H. Johnson, assistant director of the Bartol Research Foundation, has been working at the Oceanographic Institution for brief periods during the summer.

Dr. J. H. McGregor of Columbia University, left Woods Hole on Thursday after a two-day visit.

Dr. Harlan T. Stetson, director of cosmic terrestrial research at the Massachusetts Institute of Technology, recently visited Woods Hole on his vessel, the *Calypso*.

Dr. and Mrs. M. W. Bosworth, both of whom were at the Laboratory in 1940, visited Woods Hole briefly last week-end.

Among others visiting the Laboratory recently have been Drs. William F. Diller, Irene Corey Diller, H. K. Hartline, I. M. Korr, Felix Bernstein, Ernst Fischer, and D. Eugene Copeland.

Dr. Robert Chambers will leave Monday for New York to attend a conference on tissue culture.

Mr. Carl Alper, a student janitor at the Laboratory, injured his foot in an elevator and returned to his home in New Jersey last Sunday.

Dr. Kenneth C. Fisher has left for St. Johns, New Brunswick, to visit his father, who is ill.

Professor and Mrs. L. L. Woodruff last Sunday afternoon gave a tea at their home in Gansett for the present and former members of the Osborn Zoological Laboratory, Yale University, who are at Woods Hole this summer. About thirty were present.

Mr. Edward Chambers has received a Rockefeller Foundation grant to conduct research work at the Columbia County Department of Public Health in Hudson, New York. He and Mrs. Chambers will leave Woods Hole on Monday. Mr. and Mrs. Chambers entertained at Edgewood Monday afternoon for about seventy-five members of the Laboratory and their families with a garden party at which a dramatization of a Russian fairy tale was presented. Dr. Robert Chambers acted as narrator throughout the play.

INVERTEBRATE CLASS NOTES

Dr. Bissonnette's one-day survey of the Bryozoa was for many an introduction to this group. The lab study of the living material fixed in our minds at least some of the salient features of this group.

Though the Molluscs are no novelty, Dr. Mattox had no trouble keeping us interested in his discussions. Of particular interest in the lab work were the dissections of *Busycon* and the squid, and the study of the Pelicypod heart in its reactions to salt solutions and drugs.

Ann Weber's organization and the splendid weather proved an unbeatable combination for the picnic at Tarpaulin Cove. Some ambitious students made plans for competitive collecting teams, but every one was glad to abandon the project in favor of baseball. Several small groups spent the morning collecting or watching the birds. Seventy of us students, instructors and families made a full load for the *Nereis* and the *Winifred*. The remnant of forenoon was just sufficient time to build up an appetite for the picnic lunch of sandwiches and clams. Old friends of the lab aquaria, *Mytilus* and *Mya*, made an abundant feast. Mrs. Rankin, Randy Kielich and Bob Williams engaged in a watermelon-seed-blowing contest, in which Mrs. Rankin was the victor.

After lunch a game of M-ball was begun. To quote from the sports-columnist for the "Inverts," Johnny Osmun:

"Soon to be released through national sports writers are the rules for M-ball. Originating at and named for the M.B.L., this game is played literally and figuratively with the head. Nearly every day the varsity and occasionally the freshmen (faculty, principally) may be seen at Kahler stadium bouncing a ball from head to head. High score of nineteen uninterrupted bounces."

—Louise Gross and Bill Batchelor

SOME RECENT BOOKS IN THE BIOLOGICAL SCIENCES

Ackerman, E. A. New England's Fishing Industry. $4.00. Chicago.

Adams, N. E., and E. M. Bandow. A Study Guide for Applied Biology. $1.75. Burgess.

Alexander, G. An Outline of General Zoology. $1.00. Barnes and Noble.

Allen, P. W., and G. M. Cameron. Microbiology Laboratory Manual. $1.50. Mosby.

Arey, L. B. Developmental Anatomy (4th ed.). $6.75. Saunders.

Arnold, J. G., and T. L. Duggan. Laboratory Manual of General Biology (4th ed.). $1.50. Mosby.

Baitsell, G. A. Human Biology. $3.75. McGraw-Hill.

Baitsell, G. A. Manual of Biology (6th ed.). $2.75. Macmillan.

Baly, E. C. C. Photosynthesis. $4.75. Van Nostrand.

Beaver, W. C. Fundamentals of Biology (2nd ed.). $4.00. Mosby.

Beaver, W. C. Laboratory Outlines of General Biology (2nd ed.). $2.00. Mosby.

Bell, D. J. Introduction to Carbohydrate Biochemistry. University Tutorial (London).

Biological Laboratory, Cold Spring Harbor. Symposia on Quantitative Biology. Vol. VIII. Darwin Press.

Biological Symposia. Vol. II, $2.50. Vol. III, $3.50. Jaques Cattell Press.

Blum, H. F. Photodynamic Action and Diseases Caused by Light. $6.00. Reinhold.

Brockleshy, H. N., ed. Bulletin No. LIX: The Chemistry and Technology of Marine Animal Oils with Particular Reference to Those of Canada. $2.95. Fisheries Research Board of Canada.

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THE UTILIZATION OF AMMONIA BY *CHILOMONAS PARAMECIUM*

(Continued from page 157)

The material to be presented here is part of a comprehensive study of the carbon and nitrogen metabolism of *Chilomonas*, begun two years ago at the Harvard Medical School under Prof. A. B. Hastings and continued later at the Johns Hopkins University under Prof. S. O. Mast.

The problem of the source of energy for growth of *Chilomonas* was attacked by Burrows (*Protoplasma*, Bd. 31, S. 20-26, 1938), who used mass cultures of chilomonads grown in a solution like that shown in Table I (Solution A) and in a solution with the acetate omitted but with a gas phase containing a partial pressure of CO_2 equivalent to 12 cm. of mercury. Growth in both solutions amounted to only 5-6 thousand chilomonads/cc. and the conversion of substrates was too small to afford reliable analytical data.

Unfortunately this situation still obtains with respect to the inorganic solution, but as shown by Hutchens (*J. Cell. and Comp. Physiol.*, V. 16, pp. 265-267, 1940) (and unpublished results) by use of a solution to which thiamin and iron are added populations up to 1 million cells/cc. can be obtained (Table I, Solution B). In addition to the B_1 and iron it will be noted that the buffering capacity of the solution has been increased. In addition to this it is necessary to add acetic acid from time to time to neutralize the excess base left by removal of acetate and to afford additional carbon supply.

Table I.

Composition of Solutions Used for Growing Chilomonas

Substances	mg. % Solution A	Solution B
$CH_3COONa \cdot 3H_2O$	249	249
NH_4Cl	46	46
$(NH_4)_2SO_4$	10	10
K_2HPO_4	20	150
$MgCl_2$	1	1
$CaCl_2$	1	1
$FeCl_3$	—	0.17
Thiamin hydrochloride	—	0.01

In contrast with the old solution in which an inoculum of about 500 cells/cc. resulted in a final population of 5-6 thousand cells/cc. or a ten-fold increase in cell numbers, in this solution an inoculum of 500-1000 cells/cc. results in a final population of as much as 1 million cells per cc. or an increase in cell numbers of 1000 times. Thus large numbers of cells, milligrams or even grams of material are available and the amounts of substrates used are readily measurable.

In experiments which I shall not have space to present it has been found that the chilomonads utilize large amounts of acetic or lactic acids, apparently sufficient to account for all of the oxygen consumed and to furnish energy for the growth obtained in media containing these substances. The present experiments deal with the heretofore unanswered question of the possible obtaining of additional energy by oxidation of ammonia.

The experimental methods used are as follows: The organisms were grown in sterile, pure, mass cultures in 125 cc. Erlenmeyer flasks containing 50 cc. of the solution shown in Table I (Solution B). Inocula of 1-2 thousand organisms/cc. were used, transfers being made from cultures 48 hours old. Such organisms show no lag phase of growth, and all of the data presented deal with transformations occurring during the logarithmic phase of the growth curve.

The chilomonads used were from the Hopkins stock which has been maintained in sterile pure cultures since 1933. There have been two interruptions in the work; consequently the data presented were obtained using three different samples of chilomonads. No differences were noted in the growth of the various samples.

Numbers of cells were ascertained by fixing the cells in Lugol's solution and counting them in a hemacytometer. Wet and dry weights were obtained by packing the cells in centrifuge tubes terminating in capillaries, withdrawing the supernatant solution, and weighing the cells before and after drying at 110°. Total nitrogen in the organisms was measured either directly by aerating

Table II.

Wet and Dry Weights of Chilomonads During the Logarithmic Phase of Growth
(48 *Hour Cultures*)

Wet Weights mg./10^6 cells	Dry Weights mg./10^6 cells
2.64	0.738
2.42	0.559
2.42	0.563
2.60	0.692
2.51	0.530
2.52	0.595
2.48	0.575
2.49	0.563
2.87	0.767
2.11	0.545
2.51	0.567
2.48	0.567
average = 2.51	average = 0.605

Dry weight = 24% of wet weight.

the ammonia from an alkalized suspension of cells and subjecting the residue to Kjeldahl digestion, or differentially by measuring the ammonia present before and after Kjeldahl digestion, both methods yielding essentially the same results. The micro-Kjeldahl procedure of Folin was used routinely, and the amounts of ammonia were measured by determining the absorption of Nesslerized solutions at 400 millimu with a Coleman monochromater spectrophotometer. Tests for nitrite were made with sulfanilic acid - α naphthylamine and tests for nitrate with diphenylamine and diphenylbenzidine. Amide nitrogen was estimated by the increase in ammonia nitrogen on subjecting the organisms to three-hour hydrolysis in 0.5 N H_2SO_4.

The results of the investigation are as follows: Table II shows the average weight of the chilomonads during the rapid growth of the logarithmic phase of the growth curve. Thus an average cell would weigh 2.5×10^{-6} mg. and would be 24 percent solid. Table III shows the total nitrogen content of the organisms, and assuming all of this to be protein nitrogen, an approximation which seems to be justified by the fact that it is all removed by tungstic acid precipitation, the protein accounts for about 16 percent of the wet weight or 67 percent of the dry weight. Incidentally the carbohydrate accounts for about 30 percent of the solids, so these two components account for almost all of the solids.

The question to be answered, however, is whether the nitrogen in the cells accounts for all of the nitrogen utilized. Table IV gives the answer to this question. Choosing one example from these experiments (Experiment 2), which are all essentially the same, we find that the original total or ammonia nitrogen in the solution was 149γ/cc., that added in the organisms being insignificant. After 48 hours' growth 124γ/cc. of ammonia nitrogen remained in the solution; i.e. 25γ/cc. had disappeared. Subjection of the total solution, i.e. solution plus organisms, to Kjeldahl digestion gave a final total nitrogen of 149γ/cc. or 100 percent recovery. The population of the culture at this time was 4×10^5 organisms/cc. or 1 mg. of wet cells/cc. As seen from the previous table, these would be expected to be 2.5 percent N, i.e. to contain 25γ of nitrogen, which just accounts for the 25γ of nitrogen which disappeared from the solution.

We can also answer the question concerning the oxidation of ammonia, both indirectly by the fact that all of the utilized ammonia appeared as organic nitrogen and by direct analysis for nitrite and nitrate. We see that no nitrate (less than 0.025γ/cc.) could be found before or after growth of the culture and the small increase in nitrite represents less than 0.1 percent of the nitrogen used. You will note that this experiment is the only one in which this increase occurred. Therefore in the most unfavorable experiment the increase in oxidation products of ammonia is insignificantly small. The conclusion therefore is that in the solution used *Chilomonas* does not oxi-

Table III.

Nitrogen Content of Chilomonads During the Logarithmic Phase of Growth

mg. N/10^6 cells	mg. protein/10^6 cells (N $\times$ 6.25)
0.056	0.35
0.071	0.44
0.060	0.38
0.066	0.41
0.063	0.39
0.066	0.41
0.063	0.39
0.063	0.39
average = 0.064	average = 0.40

Nitrogen (% of wet weight) = 2.5%
Protein (% of wet weight) = 16%

Table IV.

Changes in Nitrogen Content of Cultures of Chilomonas

Expt.	Original NH_3-N	Final NH_3-N	Final Total N	Organic N	% of Total N Fixed	Original NO_2-N	Final NO_2-N	Original NO_3-N	Final NO_3-N	% recovery
	γ/cc.	γ/cc.	γ/cc.	γ/cc.		γ/cc.	γ/cc.	γ/cc.	γ/cc.	
1	146	128	146	18	12.3	0.01	0.01	no analysis		100
		124	149	25	16.8	0.01	0.03	—	—	100
2	149	124	149	25	16.8	0.01	0.03	—	—	100
		124	149	25	16.8	0.01	0.03	—	—	100
		113	146	33	22.6	0.01	0.01	—	—	100
3	146	112	146	34	23.3	0.01	0.01	—	—	100
		112	148	36	24.5	0.01	0.01	—	—	101
4	150	119	149	30	20.0		no analyses			99
5	147	115	146	31	21.1					98
		114	147	33	22.4		no analyses			100
		109	149	40	26.9	0.01	0.01	—	—	99
		110	150	40	26.7	0.01	0.01	—	—	99
6	151	110	150	40	26.7	0.01	0.01	—	—	99
		110	151	41	27.2	0.01	0.01	—	—	100
		110	150	40	26.7	0.01	0.01	—	—	99

dize significant amounts of ammonia, but all that it utilizes is incorporated in organic compounds.

Finally, to avoid leaving the impression that the problem is solved, I shall present the results of a part of the work on fractionation of the organic nitrogen. It would be of great interest to know just what nitrogen-containing compounds are made from the ammonia. Table V shows the results of the first attempts to characterize the proteins. Analyses have been made for amide nitrogen. You will see that this amounts to 25-30 percent of the organic nitrogen. All of it is in the tungstic acid precipitable fraction. This indicates a high proportion of dicarboxylic amino acids in the proteins and I should like to point out that of the common proteins those from grains contain amide nitrogen of this order of magnitude. It seems to me very interesting to find this situation in a starch forming flagellate.

Table V.

Amide Nitrogen Content of Chilomonads

Experiment	Organic N mg.	Amide N mg.	%
1	33	11	33
2	31	8	26
3	18	5	28
4	79	20	25
	72	18	25
5	40	12	30
6	51	12	24
7	87	23	32
		average =	28.2

Summary

1. As has been previously reported by numerous workers ammonia is a satisfactory source of nitrogen for *Chilomonas*. It has finally been possible to obtain sufficient growth of the organisms to show by direct analysis that the nitrogen found in the organisms came from the ammonia in the culture solution.

2. It has been shown that in the solution used no significant amounts of ammonia are oxidized to furnish energy for growth.

3. Finally it has been shown that of the nitrogen in the proteins a relatively high proportion is in the form of labile amide groups. Further identification of the various nitrogen containing compounds is certainly desirable.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 12.)

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FURTHER IMPLICATIONS OF FLEXIBLE PROTEIN FRAMEWORKS

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Professor at Amherst, Smith and Mount Holyoke Colleges

In this short report, it is my purpose to emphasize two sets of implications following from the flexible protein framework picture of cytoplasmic and membrane structures. I refer to (1) the synthesis of proteins and (2) the function of metallic ions in physiological processes.

(1) I have suggested that long threads or rods of (globular) native protein units in linear arrays, linked by multiple units to form frameworks, account very satisfactorily for high water/protein ratios (*Cold Spring Harbor Symposium*, 1941, forthcoming); may be capable also of accounting for the anomalous properties of cytoplasm; and that fabrics of such units may be the basic structural units in biologically active membranes (Collecting Net, *16*, 121, 1941). In such cases, the disengaged faces of the native protein units (i.e. those not in use as interlinks of the frame work) are available as templates for synthesis and other reactions. Evidently cases in which high metabolic activity is associated with low cytoplasmic viscosity (Preston, *Nature, 147*, 710, 1941) here find explanation and interpretation, for the longer the threads, the lower the viscosity and the larger the template area. The process of protein synthesis may be visualized as taking place by means (*Continued on page* 190)

NATURE, FUNCTION, AND DISTRIBUTION OF THE PHOSPHAGENS IN THE ANIMAL KINGDOM

Dr. O. Meyerhof
Research Professor of Biochemistry, University of Pennsylvania

In this place, where such a variety and abundance of living material is at our disposal, I think it worthwhile to speak about a subject, which at the same time has aspects of general as well as of comparative physiology. It is true that the investigations of the nature and distribution of the two phosphagens of the vertebrate and invertebrate muscle had their main development in the years 1927-1937 and that after that date nothing of importance was added to our knowledge. Nevertheless I venture to recapitulate before you this clear cut work on account of its bearing on different physiological topics.

About the same time, in 1927, the Eggletons in London found a labile organic phosphate in muscle, which they called phosphagen, and Fiske and Subbarow in Boston discovered the same substance, isolated it and proved that it consisted of creatine and phosphate, and named it phosphocreatine. Then rapid progress followed; it was shown that the compound was a monomolecular creatine phosphoric acid with a P-N linkage; that the hydrolysis to creatine and phosphate was intimately connected with the activity of muscle, and that in the recovery period it was resynthesized again. What seemed at first puzzling was that a great part of the creatine phosphate broken

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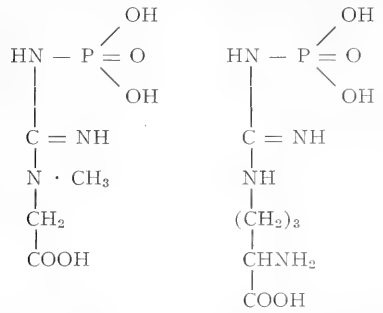

Creatine phosphoric acid

Arginine phosphoric acid

FIGURE 1.

down was already resynthesized anaerobically after contraction. Since it was shown by heat measurements that the splitting was accompanied by a positive heat of +11000 cals pro mol, apparently with a similar loss of free energy, the reversal of this splitting had to be an involuntary endothermic reaction. This puzzle could be solved very soon by demonstrating, that the synthesis was coupled with simultaneous lactic acid formation, which in itself is a strongly exothermic reaction. Under most favorable conditions two mol of creatine phosphate are synthesized for one mol lactic acid formed from glycogen. In this way the whole coupled reaction including the heat of neutralization of lactic acid is rather thermoneutral.

Before going into more detail of the coupling I may describe the simultaneous development of this problem in the direction of comparative physiology. The Eggletons found phosphocreatine in all classes of vertebrate muscle, but found no phosphagen at all in invertebrate. The authors used the method to follow the time course of color development of molybdene blue after molybdene sulfuric acid and a reducer had been added to the solution in question. While with inorganic phosphate the intensity of the color rises steeply in the first minutes, the color development in presence of creatine phosphate is much delayed for 20 minutes. In this way they showed that the bulk of the phosphate in fresh muscles of vertebrates consists of the labile organic phosphate, called phosphagen; by the same method they missed this phosphagen in invertebrate without exception.

In the year 1927 Dr. Lohmann and I took up the same problem, using muscles of crayfish. Since previously we had established the highly conspicuous role which the breakdown and synthesis of phosphocreatine plays in muscle contraction, we were convinced that some substitute must exist in invertebrate muscle and by a good chance we came immediately on the track of it. One day I had left a series of acid filtrates of crayfish muscles on my laboratory table and preferred to go to lunch instead of working them up. Returning two hours later on I found to my surprise that by this unintentional acid incubation was liberated a similar amount of phosphate, as in frog muscle filtrates by the procedure of Eggleton. So another labile substance was present, a little more acid stable then phosphocreatine. The isolated substance proved to be arginine phosphoric acid.

The peculiar constitution of the phosphagens, containing a P-N linkage, was established by comparison with a synthetic product, aminophosphoric acid. The latter behaved quite analogously with regard to stability, titration curve and heat of hydrolysis, which amounted here to +15000 cals pro mol. While the constitution of the phosphagens was established since 1928, the chemical synthesis of creatine phosphoric acid was accomplished by Zeile only in 1937 and thereby the configuration was definitely confirmed.

The first experiments proved the presence of arginine phosphoric acid in crustacean muscle only; but in 1928, working at the Zoological Station in Naples, I found it present also in representatives of annelids, molluscs and echinoderms. On the other hand, the acrania amphioxus, which is considered the immediate predecessor of the vertebrata, contained only creatine phosphate, no arginine phosphate. Therefore I spoke of a genetic "chemical mutation" of the phosphagens occurring on the level of the chordates. Indeed, arginine can be regarded as the more primitive substance and creatine as a special derivative of it, since arginine is already a component of protein. As we know now from Schoenheimer's work with isotopic nitrogen, creatine is formed in the animal body out of arginine, glycine and methionine. In contrast to arginine, creatine is an exceedingly stable substance and is not decomposed at all in the body but only dehydrated to creatinine. Finally, creatine phosphate seems better adapted to its biological purpose, since the heat of hydrolysis is 20% greater than that of arginine phosphate, so that the energy, useful for muscle work, is correspondingly greater. This speaks in favor of a biochemical improvement by substituting creatine for arginine.

Some years later, in 1932, Joseph Needham and a large group of coworkers in Cambridge, England, took up this problem of chemical mutation, enlarging the investigations to many more classes of animals. They missed all phosphagen in protozoa and coelenterates, but they found arginine phosphate in all phyla of invertebrates where muscle tissue existed. On the other hand these

animals did not contain creatine phosphate, which was already known for the majority of them. But there were two interesting exceptions: the hemichorda *Balanoglossus,* an earlier class of chordates than the more recent acrania amphioxus, contained arginine *and* creatine phosphate, and the same was true for the jaw muscles of sea urchins, the muscles in the so-called lantern of Aristotle. These muscles contained both phosphagens, about twice as much arginine phosphate as creatine phosphate. At first sight this seemed to be in contradiction to the concept of chemical mutation, but Needham takes it as confirmation of the evolutionary theory of Bateson, who looks on the Echinoidea as the immediate precursors of the primitive chordate since both have the same kind of plutei-larvae. Indeed, an ontogenetic fact serves to support Needham's interpretation: the larvae themselves contain only arginine phosphate, while creatine appears after the metamorphosis. One other exception, which is not explained or cleared up so far, was found by a pupil of Needham in 1937: the brittle stars, belonging to the class Ophiuridea in the phylum Echinodermata, seemed to contain only creatine phosphate. The present state of our knowledge is given in Figure 2 in condensed form (after Needham & Baldwin):

Phylum and class	Arginine phosphate	Creatine phosphate
Most invertebrate phyla	+	—
Echinodermata		
Crinoidea	+	—
Asteroidea	+	—
Holothuroidea	+	—
Echinoidea	+	+
Ophiuroidea	—	+
Protochordata		
Tunicata	+	—
Enteropneusta	+	+
Cephalochorda	—	+
Vertebrata, all classes	—	+

FIGURE 2.

While in general no discussion arose between the different workers in this field owing to the ease, with which both phosphagens can be distinguished one from the other and from other compounds, uncertainty prevailed for several years regarding the phosphagen of the cephalopods, like the octopus. In the first investigation in Naples I could not find any phosphagen in their mantle muscle, probably on account of the speed with which it decomposes in dissecting. Baldwin, a coworker of Needham's, claimed to have found a phosphagen which should differ in some points of arginine phosphate. Such a possibility seemed suggestive, since Japanese authors had discovered in octopus muscle a basic substance, which proved to be a condensation product of arginine and propionic acid, called octopine. This substance was thoroughly investigated and synthesized by Prof. D. Wright Wilson in Philadelphia. But, as was proved by Dr. Lohmann in our Laboratory, Baldwin was led astray by technical circumstances and the isolated phosphagen of octopus proved to be normal arginine phosphate. This was confirmed by Dr. Wilson, who showed, moreover, that fresh mollusc muscles, of octopus or of scallop, contain mostly arginine, and that octopine is formed post mortem, but probably by an enzymatic condensation, the significance of which is unknown.

I shall now discuss the fact that the specificity of the two phosphagens extends also to the enzymes concerned with their turnover. Therefore I must come back to the coupled reactions, in which the phosphagens take part. Already at the beginning of this paper I stated that in muscle activity phosphocreatine breaks down before lactic acid is formed; that the energetic role of lactic acid formation consists in the resynthesis of creatine phosphate, a process of anaerobic recovery. This connection became especially clear by the discovery of Einar Lundsgaard in 1929 of the "alactacid contraction" by poisoning the muscle with iodoacetic acid. In such a muscle a restricted amount of work can be done anaerobically, while no lactic acid is formed. At the same time creatine phosphate breaks down exactly proportionally to the work done and no anaerobic synthesis takes place. Lundsgaard established the same relation between arginine phosphate and invertebrate muscle work by poisoning the claw muscle of the spider crab with iodoacetic acid.

In the years following these discoveries the single enzymatic reactions, by which the energy and the phosphate are transferred from carbohydrate metabolism to creatine, were cleared up. Without going into details, I may state, that these

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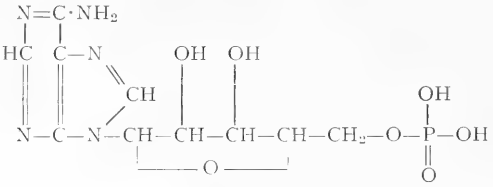
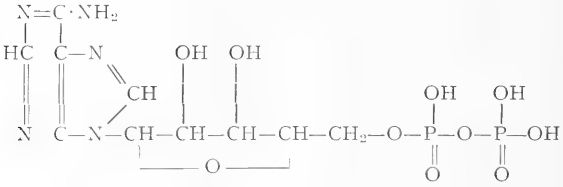
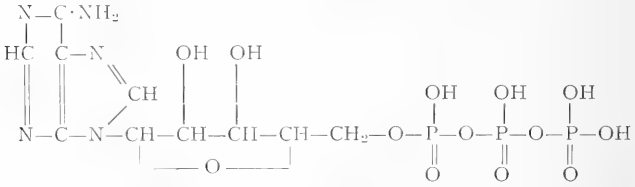


I. Adenylic acid = Adenosinmonophosphoric acid



II. Adenosindiphosphoric acid



III. Adenylpyrophosphoric acid = Adenosintriphosphoric acid

FIGURE 3.

reactions are so called "transphosphorylations," where the phosphate group is transferred from one compound to another without becoming intermediarily inorganic phosphate. All these transphosphorylations proceed by means of one single system, the adenylic system, which I show in Figure 3. It consists of three steps, discovered by Dr. Lohmann in the Heidelberg Institute. This system is to be regarded as phosphorylating coenzyme. Together with different proteins as carriers it is responsible for phosphorylations, dephosphorylations and transphosphorylations in muscle metabolism.

The breakdown of phosphocreatine proceeds for instance, as Lohmann showed, in the way of equation A and B of Figure 4. On the other hand, creatine phosphate is synthesized by coupling with two different steps of sugar metabolism. One of these is explained by equations C and D. The last phosphorylated intermediary in lactic acid formation is phosphopyruvic acid. The latter forms pyruvic acid by transphosphorylation with adenylic acid. Adenosintriphosphate transfers its phosphate group to creatine.

A careful study by Dr. Lohmann revealed that the adenylic system is exactly the same in invertebrate as in vertebrate muscle, only the enzyme proteins are of different stability, and in crab muscle the step to adenosindiphosphate is easier than to adenylic acid (E). On the other hand the enzymes concerned with the phosphagens are specific: an enzymatic extract of frog or rabbit muscle phosphorylates creatine in presence of adenylpyrophosphate but not arginine, and arginine

phosphate is stable in it whereas creatine phosphate is split. The opposite is true for extracts from crayfish. Here only arginine reacts. Needham and his collaborators used this method of Lohmann's to reinvestigate in 1937 their former findings of the distribution of phosphagens in the muscle of Echinoderms. While in general the enzymatic muscle extracts of invertebrates could only react with arginine, the jaw muscles of sea urchins gave enzymatic extracts, which were able to phosphorylate creatine as well as arginine; they contain both sets of enzymes. On the other hand the holothurian, also an echinoderm, which possesses only arginine phosphate, gives enzymatic extracts, active towards arginine but inactive towards creatine. These experiments give a striking confirmation of the result that in the jaw muscles of Echinoids, both phosphagens are present as active functioning substances.

I may add some words about the distribution of the phosphagens in ontogenesis. According to Needham and his school the developing embryo contains an amount of phosphagen, which is roughly proportional to the amount of muscle tissue in the same stage of development. But muscle is not the only tissue to contain phosphagen. Gerard and his coworkers have found phosphagen in the peripheral nerves, about 1/4 as much as in the same quantity of muscle; part of this phosphagen breaks down in stimulation and more in prolonged asphyxia. While in mammalian nerves the phosphagen was creatine phosphate, in lobster nerve, as was to be expected, it was arginine phosphate.

Still more interesting is the high content of phosphocreatine in the electric organ of fishes. The percentage content in the organ of *Torpedo* is not much less than in frog muscle and related to the dry weight of the organ may be even higher. The same phosphorylating enzymes as in muscle extract were found in the extract of the electric organ. The evidence that the phosphagen breaks down in connection with the electric discharge is so far rather incomplete. This is an interesting question, in view of the findings of Dr. Nachmansohn, that the formation and the disappearance of acetylcholine are intimately connected with the electric discharge and that with regard to the concentration of cholinesterase the electric organ corresponds to the nerve endplate and not to the muscle fiber. Nevertheless, also in muscle activity the breakdown of phosphagen apparently is not the first chemical process unchained by stimulation and immediately responsible for the contraction, but one of the consecutive steps which follow this unknown fundamental reaction. Therefore the role of creatine phosphate in the electric organ may be a similar one. Finally, as was found by Torres in the Heidelberg Institute, mammalian spermatozoa possess a high content of phosphocreatine and a high content of enzymes, to synthesize it. Together with other observations about anaerobic glycolysis and movement of spermatozoa after poisoning with iodoacetic acid this observation favors the view that the same chemical reactions are responsible for the movement of spermatozoa as for the muscular movement.

A) 2 creatinephosphate + adenylic acid $\rightleftharpoons$ 2 creatine + adenosintriphosphate

B) adenosintriphosphate $\rightarrow$ adenosindiphosphate + H_3PO_4 $\rightarrow$ adenosinmonophosphate (adenylic ac.) + 2 H_3PO_4

Sa. 2 creatinephosphate $\rightarrow$ 2 creatine + 2 phosphate

C) 2 phosphopyruvic ac. + adenylic ac. $\rightarrow$ 2 pyruvic ac. + adenosintriphosphate

D) adenosintriphosphate + 2 creatine $\rightarrow$ adenylic ac. + 2 creatinephosphate

E) adenosintriphosphate + arginine $\rightleftharpoons$ adenosindiphosphate + argininephosphate

FIGURE 4.

Summarizing our present knowledge, we may say, in short, that creatine phosphate developed out of arginine phosphate on the evolutionary level of the late echinoids and the early chordata and that both phosphagens have exactly the same function, which in muscle is undoubtedly the transfer of chemical energy by means of transphosphorylation and which may be similar in other irritable tissue.

With growing insight into the biochemical structures of the cell surely more examples of such mutations will be discovered. So far I know only one other, studied by Dr. Wald. He found that fresh water fishes possess a kind of visual purple different from that of other animals, a visual purple, called porphyropsin, not derived from vitamin A, like the normal purple, Rhodopsin, but from a homologue, vitamin A_2. Also here the physiological function of the chemically mutated substance must be essentially the same as that of the original substance. We may hope that by artificially induced chemical mutations in strains of virus, we may learn still more about the meaning of these genetic chemical transformations.

(This article is based upon a lecture presented at the Marine Biological Laboratory on August 22.)

ELECTRICAL POTENTIAL AND ACTIVITY OF CHOLINE ESTERASE IN NERVES

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Electrical changes during nerve activity occur within a few milliseconds or even within a fraction of a millisecond. Chemical reactions connected with these changes must have approximately the same rapidity. This time factor is of primary importance for the theory that acetylcholine (ACh) might be the "mediator" of nerve impulses across ganglionic synapses and neuromuscular junctions. Such a substance must appear and disappear with the speed of the electrical phenomena.

No data are available which establish the rate of ACh appearance during nerve activity. But the possible rate of its removal at motor end plates and synapses has been determined. ACh is inactivated by the specific enzyme choline esterase. Studies on the concentration and distribution of choline esterase have revealed that at motor end plates and ganglionic synapses as well as at synapses of the C.N.S. considerable amounts of ACh can be split in milliseconds. These amounts if liberated would have a stimulating action. The experiments therefore indicate that the removal of ACh can occur at a rate rapid enough for the assumption that ACh is involved in the transmitter process.

These results, however, do not imply that ACh is the synaptic transmitter as originally conceived. Recent investigations suggest that the theories of Loewi and Dale must be altered to account for the ACh metabolism which closely parallels the electrical changes occurring everywhere at or near the neuronal surface. This new conception is based on two lines of observations.

(1) The first argument is based on investigations carried out on the electrical organs of fishes. In spite of the great power of the discharge in these organs there is no reason to regard the electricity of these organs as extraordinary compared with that of ordinary nerves. The organ is formed by electric discs or plates which are arranged in series. It is only this arrangement in series by which these organs are distinguished from other excitable structures and by which the high E.M.F. is attained.

In strong electric organs (*Torpedo* and *Electrophorus electricus* (Linnaeus)) exists a high concentration of choline esterase. These organs can split in 60 minutes an amount of ACh equivalent to 1-3 times their own weight. The essential point is the fact that in these organs considerable amounts of ACh can be split during the refractory period which is of the order of milliseconds. This makes possible the assumption that ACh is closely connected with the discharge. The prerequisite for such a conception is the possibility of a quick removal of the active substance. The high concentration of the enzyme appears particularly significant in view of the high water (92%) and low protein content (2-3%) of the organs.

In the weak electric organ of rays the enzyme concentration is low. If in the three species number of plates per cm. and E.M.F. per cm. are compared with the concentration of choline esterase a parallelism, within certain limits, is obtained. This parallelism has also been demonstrated, with Coates and Cox, on the electric organ of *Electrophorus electricus*. If the number of plates per cm. and the E.M.F. per cm. are determined from the head to the caudal end, an S-shaped curve is obtained. The curve obtained for the concentration of choline esterase is essentially the same. Electric organs are highly specialized in their function. The discharge is here the final event. There is no question of a transmitter function as there is no second unit to be stimulated. The fact that a specific enzyme is so highly concentrated in this organ—so poor in protein—is in itself support for the assumption that the substrate is connected with its function. The parallelism found between intensity of discharge and activity of the enzyme emphasizes this relationship.

It could moreover be demonstrated with the electric organ of *Torpedo marmorata*, with Fessard and Feldberg, that ACh is released during the discharge and that injection of ACh into the organ produces a discharge. The discharge is greatly enhanced if the choline esterase is inactivated by eserine whereas eserine itself has no effect.

The second line of observations leading to a modification of the original theories is the localization of the enzyme inside the nerve cell. Only a quantitative difference exists between the concentration of the enzyme in nerve fibres and that at synapses. Experiments on the superior cervical ganglion of cats suggested that the difference is related to a concentration of the enzyme at or near the surface of the nerve cell and therefore high at synaptic regions where the endarborization increases the surface. Direct evidence for this assumption was offered last year here in Woods Hole with Dr. Boell with experiments on the giant fiber of squids. It was found that practically all the enzyme is localized in the sheath and that only negligible amounts occur in the axoplasm. This localization indicates that ACh metabolism occurs not only at synapses but everywhere at or near the surface. The difference is

only a quantitative one. Bioelectric potentials are surface phenomena. The localization of the enzyme at or near the surface is therefore particularly pertinent in view of the parallelism between voltage and enzyme activity.

The observations suggest that ACh is intrinsically connected with the electrical changes occurring during nerve activity at the neuronal surface. Thus the controversy between "electrical" and "chemical" theory of transmission of nerve impulses becomes meaningless. Both are signs of the same event. According to Eccles and Sherrington and Lorente de Nó the excitable properties of central neurons are similar to those of the peripheral axons. Gasser and Erlanger scrutinizing the whole problem at the symposium on the synapse arrived at the conclusion that conduction of nerve impulses along fibers and across synapses is essentially the same process, and that the difference is only a quantitative one. This view is not compatible with the theory of a specific synaptic transmitter. The new conception agrees well with these conclusions based on the electrical phenomena.

How can a relationship be pictured between voltage and action of ACh? According to the equation $V=E-IR$ (where V is = voltage, E = E.M.F., I = current and R = resistance) two assumptions can be made about the way in which ACh may act:

1) ACh can produce the E.M.F. directly by action on the surface. This possibility appears to be the less probable. There is a great difference in concentration of strong ions between the inside and the outside of the nerve fiber. It seems more probable that these differences are responsible for the potential differences.

2) ACh can decrease the resistance and this again by action on the surface, for instance by increasing the permeability. This way can be easier conceived. The resistance decreases during nerve activity, as shown by Cole and Curtiss. The action of ACh may be connected with this transient change of resistance. A substance which is released and can be removed within a millisecond could well account for such a quickly reversible alteration of the membrane. It would be compatible with the ideas on propagation of nerve impulses as developed by Keith Lucas and Adrian.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 19.)

CHEMICAL COMPOSITION OF MITOCHONDRIA AND SECRETORY GRANULES

Dr. Albert Claude

The Rockefeller Institute for Medical Research, New York, N. Y.

The chemical nature of mitochondria, and the function which they assume in the economy of the cell, are problems which have failed to be solved by cytological techniques. The experience of the past fifty years leaves little hope that definite information can be gained unless we find ways of isolating these cytoplasmic elements and submit them to direct chemical analysis and biological tests. Recent results indicate that mitochondria can be separated from the other components of the cell by means of a simple method of differential centrifugation at high speed. Under proper conditions, other particulate components of cytoplasm, especially the relatively large elements known under the names of plasts, secretory or zymogen granules, can be isolated by the same technique.

In previous studies, small particles, ranging in size between 60 and 200 mμ diameter were isolated from normal and tumor cells. Chemically, these tissue particles are complexes made up essentially of phospholipids and ribonucleoprotein. Small particles of this type appear to be general constituents of cells and there is evidence that the elements purified in the high speed centrifuge represent mitochondria, or fragments of mitochondria (A. Claude, *Science, 90,* 213, 1939; *Symposia on Quantitative Biology,* Vol. *9,* Cold Spring Harbor, 1941; R. R. Bensley and N. L. Hoerr, *Anat. Rec., 60,* 449, 1934). In addition to mitochondria and "secretory" granules, the cytoplasm of normal cells appear to contain also particulate elements of smaller size which would escape detection in the ordinary microscope.

In the present work three different kinds of granules were obtained from the cytoplasm of liver cells. Liver tissue from guinea pigs was extracted with four times its weight of neutral water. Free nuclei and cellular debris were removed by a preliminary centrifugation at low speed. Further fractionation of the liver extract was carried out in a high speed centrifuge, under a uniform centrifugal force of 18,000 × gravity. The first fraction was brought down by a run of exactly 5 minutes at high speed. Under the microscope, this purified fraction was found to consist of small spheres of various sizes, ranging approximately from 0.5 to 3μ, and resembling fat globules. On chemical analysis, these granules were found to contain 12 per cent nitrogen, 0.9 per cent phosphorus, and 53 per cent carbon. Twenty-two to 24 per cent of the material was soluble in alcohol and chloroform. In size and appearance, the large granules correspond to the "secretory" granules, or plasts, which can be demonstrated in the cytoplasm of the hepatic cell

by proper cytological techniques (R. Noël, *Arch. Anat. Micros., 19,* 1, 1923).

The second fraction was sedimented by 45 to 60 minutes centrifugation at high speed. The purified material was a jelly-like substance, colorless and entirely transparent. It was composed of small particles visible, under dark-field illumination, as dense, refractile bodies, spherical in shape, or slightly elongated. The results of chemical analysis were 9 per cent nitrogen, 1.2 per cent phosphorus and 56 per cent carbon. Forty to 45 per cent of the material was soluble in organic solvents. Physically and chemically, the second fraction corresponds to the usual "mitochondria" fraction referred to above. The third fraction was sedimented by a run of two hours at high speed. The purified pellet was a perfectly transparent mass, cherry-red in color. In the dark-field microscope, a suspension of the material was found to be composed of small particles which, from their sedimentation rate, appear to range in size between 40 and 60 mμ diameter. A ribose nucleic acid was isolated from both the first and the second fractions. This nucleic acid was identified by characteristic absorption spectrum in the ultraviolet, and typical color tests.

The above observations indicate that the "secretory" granules of the guinea pig liver, like the small tissue granules, are complex formations made up of lipids and proteins. Both the secretory granules and the mitochondrial material contain a ribose nucleic acid in about the same proportion. Both elements are equally sensitive to acid. Slight acidification of the medium causes rapid agglutination of the granules or the particles, and in both cases, a point of minimum solubility is found at pH 3.5. The differences brought out by elementary analysis are of a quantitative nature, and, to a great extent, result from the fact that the large granules contain 20 per cent lipids, against 40 to 45 per cent for the small particles.

The similarity in chemical constitution and behavior between the large granules and the small particles suggests a possible continuity between these cytoplasmic elements. The findings may give support to the view that mitochondria develop into secretory granules, as suggested for the liver by Noël (*Arch. Anat. Micros., 19,* 1, 1923). If this is the case, we should be able to isolate forms of transition between mitochondria and mature secretory granules. This point will be the matter of further work.

An important problem intimately connected with the chemical nature of the cytoplasmic granules is the role they may play in cellular physiology. In this respect, it may be significant that iron and copper are found in relatively large amounts in secretory granules and mitochondria. The particles isolated from the dried cells of Brewer's yeast had a copper content of 0.116 per cent. In this case, the purified pellet presented a definite blue color, resembling that of hemocyanins. Similar fractions from other sources were found to contain copper also, but in lesser amounts. The proportion of copper was 0.023 per cent for the particles derived from a mouse leukemia, 0.016 per cent for the small particles of the guinea pig liver, and as much as 0.034 per cent for the secretory granules. This latter quantity is not negligible if we consider that it represents approximately 20 per cent of the copper content of the hemocyanin of *Limulus* (A. C. Redfield, *Biol. Reviews, 9,* 175, 1934). There is evidence tthat the copper present in the granules occurs in combination with a protein. The possibility that the copper compound, associated with the phospholipoid-ribonucleoprotein complex, may act as a catalyst of respiration is being investigated.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 19.)

INVERTEBRATE CLASS NOTES

The study of Echinoderms and Arthropods, two field trips, beach parties, and a baseball game were the high lights of another "typical" week for the Inverts.

The behavior of starfish, the pretty if complex "Aristotle's Lantern" of the sea urchin, regeneration in the sea cucumber, particularly held our interest. Dr. Bissonnette's lecture covered the taxonomy of the Echinoderms, their unique organ systems, their strange development. Dr. Martin has been following a similar plan in presenting the Arthropods. In addition to the lobster and crab, we have been studying specimens of local crustacea, autotomy in the fiddler crab and its color reactions.

The field trips to Hadley Harbor and North Falmouth were most fruitful, both in view of the number and variety of specimens. The exhibit in the lobby of the Brick Building will testify to that. The North Falmouth trip was a chilly one. Even the shoulders could scarcely keep warm. The hot coffee went quickly at lunch. The afternoon was warmer, the collecting, appropriately for the last trip, was the best ever.

Our versatile group displayed their talents at the "Four O'Clock Club" in Falmouth. Julie and "Stubby" Rankin thrilled the audience with their dancing. Not to be outdone, Jack Osmun, Sid Pond, Howie Miner and Bob Corder sang their favorites, after which John led the enthusiastic patrons in group singing.

The Staff-Invert baseball game was great fun. Dr. Martin is some pitcher. The game was close

(Continued on page 191)

PAPERS AND DEMONSTRATIONS PRESENTED AT THE GENERAL SCIENTIFIC MEETING, 1941

Tuesday, August 26, Morning Session, 9:00 A. M.

CASWELL GRAVE: Further studies of metamorphosis of Ascidian larvae.

CASWELL GRAVE: The "Eye Spot" and light responses of the larva of *Cynthia partita*.

LLOYD BIRMINGHAM: Regeneration in the early zooids of *Amaroucium constellatum*.

IVOR CORNMAN: Characteristics of the acceleration of *Arbacia* egg cleavage in hypotonic seawater.

ETHEL BROWNE HARVEY: Material inheritanee in Echinoderm hybrids.

E. S. GUZMAN BARRON AND J. M. GOLDINGER: Intermediary carbohydrate metabolism of sperm and eggs of *Arbacia* before and after fertilization.

J. D. CRAWFORD, D. BENEDICT, A. B. DUBOIS AND A. E. NAVEZ: On contraction of the *Venus* heart.

ALFRED M. LUCAS AND JAMES SNEDECOR: Coordination of ciliary movement in the *Modiolus* gill.

LORUS J. MILNE: Preparing an animated diagram of somatic mitosis.

Tuesday, August 26, Afternoon Session, 2:00 P. M.

E. NEWTON HARVEY: Stimulation by intense flashes of ultra violet light.

T. C. EVANS, G. FAILLA, J. C. SLAUGHTER AND E. P. LITTLE: Influence of the medium on the radiosensitivity of *Arbacia* sperm.

C. LADD PROSSER AND G. L. ZIMMERMAN: Comparative pharmacology of myogenic and neurogenic hearts.

LOIS E. TEWINKEL: Structures concerned with yolk absorption in the dogfish, *Squalus acanthias*.

W. H. F. ADDISON: The distribution of elastic tissue in the arterial pathway to the carotid bodies in the dog.

RICHARD G. ABELL AND IRVINE H. PAGE: Behavior of the arterioles in hypertensive rabbits, and in normal rabbits following injection of angiotinin.

Wednesday, August 27, Morning Session, 9:00 A. M.

M. H. JACOBS AND DOROTHY R. STEWART: Catalysis of ionic exchanges by bicarbonates.

DOROTHY R. STEWART AND M. H. JACOBS: The role of carbonic anhydrase in the catalysis of ionic exchanges by bicarbonates.

MARTIN G. NETSKY AND M. H. JACOBS: Some effects of desoxycortico-sterone and related compounds on the mammalian red cell.

HERBERT SHAPIRO AND HUGH DAVSON: Permeability of the *Arbacia* egg to potassium.

A. K. PARPART: Lipo-protein complexes in *Arbacia* eggs.

S. E. HILL: Relation between the action potential and protoplasmic streaming in *Chara* and *Nitella*.

AURIN M. CHASE: Observations on luminescence in *Mnemiopsis*.

Papers Read by Title

FRED W. ALSUP: Photodynamic studies on *Arbacia* eggs.

IVOR CORNMAN: Disruption of mitosis in *Colchicum* by means of colchicine.

T. C. EVANS: The effect of roentgen radiation on the jelly of the *Nereis* zygote.

R. RUGGLES GATES: Tests of nucleoli and cytoplasmic granules in marine eggs.

RUSSELL P. HAGER: Sex-linkage of stubby (*sb*) in *Habrobracon*.

E. R. HAYES: The Elasmobranch interrenal; a preliminary note. The interrenal body of *Alopias vulpinus* (Bonnaterre).

DWIGHT L. HOPKINS: The cytology of *Amoeba verrucosa*.

GEORGE W. HUNTER, III, AND EDWARD WASSERMAN: Observations on the melanophore control of the cunner *Tautogolabrus adspersus* (Walbaum).

FLORENCE MOOG: The influence of temperature on reconstitution in *Tubularia*.

CLINTON M. OSBORN: Factors influencing the pigmentation of regenerating scales on the ventral surface of the summer flounder.

G. H. PARKER: Hypersensitization of catfish melanophores to adrenaline by denervation.

LEONARD P. SAYLES: Implants consisting of young buds, formed in anterior regeneration in *Clymenella*, plus the nerve cord of the adjacent old part.

A. A. SCHAEFFER: *Chaos nobilis* Penard in permanent culture.

VICTOR SCHECHTER: Further studies in *Mactra* egg cells.

SIDNEY F. VELICK: The effect of centrifugation upon the oxygen consumption of *Arbacia* eggs.

ALLYN WATERMAN: Ectodermization of the larva of *Arbacia*.

RALPH WICHTERMAN: Studies on *Zoochlorella*-free *Paramecium bursaria*.

FLOYD J. WIERCINSKI: An experimental study of intracellular pH in the *Arbacia* egg.

E. ALFRED WOLF, MARYON DYTCHE AND MILTON SCHAFFEL: Heat produced by respiring whole blood of *Tautoga onitis* and *Mustelus canis*.

C. L. YNTEMA: Effect of differences between stages of donor and host upon induction of auditory vesicle from foreign ectoderm in the salamander embryo.

Wednesday, August 27, 2:00 - 4:00 P. M. Demonstrations

L. F. BOSS AND M. H. JACOBS: Stabilized source of current for lamps and other purposes.

E. R. CLARK AND ELEANOR LINTON CLARK: Behavior of giant cells as observed in the living mammal.

E. N. HARVEY AND F. J. M. SICHEL: Apparatus for intense flashes of ultra violet light, and the killing of small organisms.

E. N. HARVEY AND F. J. M. SICHEL: Apparatus for high speed photography with the microscope.

KURT G. STERN: An air-driven high-speed centrifuge for optical observations.

IVOR CORNMAN: Disruption of mitosis in *Colchicum* by colchicine.

SEARS CROWELL: *Nematostella*, a simple anemone, suitable for laboratory work in general zoology.

SEARS CROWELL: The nematosomes of *Nematostella*.

JOHN KEOSIAN: Apparatus of simple construction for use in microchemical work.

JOHN KEOSIAN: A spot test method for the quantitative determination of magnesium in tenths of a microgram.

ELEANOR H. SLIFER: A mutant *Drosophila melanogaster* with extra sex combs.

LOIS E. TEWINKEL: Structures concerned with yolk absorption in the dogfish.

DR. ERIC LOEWENSTEIN: Demonstration of fluorophotometer, and discussion of fluorometric methods of determining biological substances.

DR. LAURENCE IRVING, DR. P. F. SCHOLANDER, MR. C. LLOYD CLAFF, MR. GEORGE EDWARDS, MR. NIELS HAUGAARD: Section A: Sensitive volumetric apparatus devised by Dr. P. F. Scholander, as used in the continuous measurement of respiration and in the measured delivery of small quantities of liquid. Section B: A system suitable for aquatic animals, sensitive to 0.02 cc, used to measure the O_2 consumption of fishes of 20-200 grams body weight at 10 minute intervals for periods lasting for 12 to 24 hours.

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris I. Gorokhoff and Judy Woodring.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879, and re-entered, July 23, 1938.

BOOK REVIEWS

HANNUM, C. A. "Comparative Chordate Anatomy." pp. vii + 211. Stanford University Press. 1941. $2.00.

This is one of the numerous texts of comparative anatomy, based on the author's own course, which have appeared in the last year: in the opinion of the reviewer, it is better than most. Too many texts of this type stop short at birds, on the ground that any mammal, all too frequently the cat, should be the subject of a separate semester's work. Hannum has the courage to put the mammal where it belongs: in the last chapter, not in the next book. This makes it possible to give a one year survey of the animal kingdom, offering the student an adequate background for his subsequent specialised studies—among them, should it be desirable, the cat.

This book not only describes the dissection of the usual laboratory types in clear language but also discusses classification, phyletic origins and ontogenesis. All anatomical terms are italicised and the author holds a happy balance between the English and Latin tongues. The absence of illustrations and the paper binding are not defects for they keep the price within the limit which any student can be asked to pay; and for his money he will get heavier paper and clearer printing than he might expect.

There is no doubt that this text deserves careful consideration from every teacher who is planning next year's course in comparative anatomy.

Peter Gray

STILES, K. A. "Handbook of Microscopic Characters of Tissues and Organs." pp. vi + 148. Philadelphia, The Blakiston Company. 1940. $1.50.

This work endeavors to apply to the identification of tissues the principles used by taxonomists in the construction of "keys". It is very doubtful whether anyone not intimately acquainted with the taxonomy of a group has ever successfully identified a species from a key: certainly the student using this book will require the help of a competent and willing instructor. In his Preface the author states that the book "is actually an abbreviated text which contains . . . the fundamentals of regular histology textbooks, but in a form much more easily and quickly grasped by the reader": that "to the student preparing for State and National Board Examinations, it is invaluable": that it "serves primarily to alleviate the welter of confusion which arises when one is presented with a mass of facts and not enough basic knowledge of the subject matter to make an intelligent choice". The reviewer cannot go all the way with the author in his opinion of the text but it will doubtless be found useful by those who desire a condensed concomitant to their regular lecture and laboratory volumes.

The few illustrations for which space has been found are excellently drawn and the tabular summaries which preceed each section could not be improved. The book is ring bound with a waterproof cover and interleaved with blank sheets.

Peter Gray

ACADEMIC RANK OF M. B. L. INVESTIGATORS

The number of investigators of each academic rank registered (filled out blanks before August 21) at the Marine Biological Laboratory:

Professors	66
Associate Professors	23
Assistant Professors	42
Instructors	35
Research Associates	7
Assistants	55
Fellows	20
Graduate Students (not listed elsewhere)	37
Medical Students	5
Undergraduate Students	8
Preparatory Students	5
Not falling in above categories	26

SEMINARS AT MOUNTAIN LAKE

Recent speakers at the seminars, with their topics, at the Mountain Lake Biological Station have been:

Grace T. Wiltshire, "Nesting Habits of Alleghanian Warblers."

Edward M. McCrady, Jr., "Physiology and Embryology of the Opossum," and "Leonardo de Vinci as an Anatomist."

Robert K. Burns, "Further Notes on the Embryological Development of the Opossum."

H. Eugene Brown, "Progress Report on Termite Investigations."

T. S. Painter, "Salivary Chromosomes."

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
August 31	11:39	.....
September 1	12:24	12:42
September 2	1:25	1:43
September 3	2:23	2:39
September 4	3:14	3:30
September 5	4:01	4:18
September 6	4:42	5:00
September 7	5:23	5:42
September 8	6:05	6:25
September 9	6:45	7:09

ITEMS OF INTEREST

The invertebrate course of the Marine Biological Laboratory is holding its last session this morning.

Dr. Colin M. MacLeod, associate of Rockefeller Institute, has been appointed director of the Bacteriological Laboratories at the New York University College of Medicine.

Dr. Frank H. Connell has been promoted from assistant professor to full professor of zoology at Dartmouth College, and Dr. James F. Crow has been appointed instructor there.

Dr. Samuel R. M. Reynolds has been appointed research associate in the department of embryology of the Carnegie Institution of Washington (Baltimore). He has been associate professor in physiology in the Long Island College of Medicine.

Recent appointments at Harvard University include those of Drs. Paul J. Allen and Roger W. Sperry as research fellows in biology. Dr. Anthony O. Dahl has been promoted to faculty instructor in biology and tutor.

Mr. William H. Burt has been promoted from instructor to assistant professor of zoology at the University of Michigan. He is also curator of mammals in the Museum of Zoology.

Misses Priscilla Anderson and B. Elizabeth Horner have been promoted to instructorships in zoology at Smith College.

Dr. Elso S. Barghoorn, Jr. of Harvard University has been appointed instructor in the biology department of Amherst College.

Mr. Sidney M. Pond, who graduated from Wesleyan this spring, and is also in the invertebrate zoology course, has been appointed graduate assistant in biology at Wesleyan.

Miss Juanita Senyard, who graduated from Oberlin College in June, has been appointed graduate assistant in histology at Mt. Holyoke College.

M.B.L. TENNIS CLUB

In the finals of the ladies' singles, D. Baitsell defeated Mary Chamberlain, 6-2, 6-0. Stunkard and Evans won over Krahl and Lancefield in the finals of the men's doubles, 7-5, 6-2. Gary Colton defeated Huntington Mavor in the junior singles, 6-2, 6-3. A cup was presented to the winner of the junior singles by Dr. D. E. Lancefield. The winners of the other tournaments will have their names engraved on cups owned by the Club.

Recent visitors at the Laboratory include Drs. H. J. Muller, Gertrude Gottschall, Oscar Bodansky, Elvira de Liee, Benjamin C. Gruenberg, Hans Gaffron, R. Leuchtenberger, R. Schoenheimer, and R. Beutner.

Among the new investigators who have recently come to the Laboratory are Dr. Robert Bloch of Yale University, who is doing research work in the library, and Miss Pauline Sullivan, teacher of biology and chemistry at the Choate School, Brookline, Massachusetts, who is assisting Dr. B. H. Grave in histological work.

Several men at the Laboratory have recently been called up by their respective draft boards: Lauren C. Gilman went into service at Camp Devens several weeks ago; Robert Spier leaves on September 15 for Camp Devens; H. Duncan Rollason, Jr., left last week for Connecticut to take his physical examination.

Mr. Elmer Higgins, chief of the Division of Inquiry respecting Food Fishes of the U. S. Fish and Wildlife Service will visit the Fisheries station in Woods Hole early in September. Mr. Higgins was director of the Bureau of Fisheries Station here for several years.

Dr. Gerald W. Prescott, who has been on the staff of botany course at the Marine Biological Laboratory for several summers, taught courses in aquatic flowering plants and the taxonomy of the fresh water algae at the biological station of the University of Michigan this summer.

Dr. Leonard I. Katzin, who worked at the Marine Biological Laboratory last year, is at present with the U. S. Public Health Service, assigned to the Southeast Health District of the State of Virginia. His assignment is on a mosquito control program.

M.B.L. CLUB NOTES

The M.B.L. Club House will remain open until approximately September 18. Tonight's dance is to be the last of the season.

Miss Margaret Mast is the new chairman of the house committee of the M.B.L. Club. The new chairman of the social committee is Mrs. Shirley D. Hobson.

In the ping pong tournament Dr. John O. Hutchens was the victor in the men's singles, defeating Richard Byrrum, and will have his name engraved on the trophy paddle. In the finals of the ladies' singles, Katya Zarudnaya defeated Marion Davis.

IN MEMORY OF DECEASED MEMBERS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

Memorials Adopted at the Annual Meeting of the Corporation, August 12, 1941

DAVID HILT TENNENT

In the premature death of David Hilt Tennent, the Corporation of the Marine Biological Laboratory has lost the crowning years in the life of a member distinguished for his accomplishment in research and even more for his quality as a man. Tennent was an investigator who proceeded without haste, yet unceasingly; for him quality not quantity of publication was the prime consideration, yet the volume of his published work is impressive. He was honored for his work by the Presidency of the American Society of Zoologists and by similar offices, and most notably by election to the National Academy of Science. He made outstanding contributions in his studies upon hybridization, fertilization and egg organization in Echinoderms, and in his later research upon photosensitization in which he took especial satisfaction since he regarded it as the most important work of his life. These contributions, which are familiar to workers in these fields, are not so well known to many investigators in other lines, because Tennent was the most modest and retiring of men. He had none of the flare for self-advertisement that carries some men so far on a modicum of worth. His every publication was marked not only by the critical nature of his observations and experiments but also by the meticulous care with which each phrase was weighed to make sure it meant exactly what he had in mind, no more and no less. What he wrote or said publicly was always as exact as he could make it. Knowing the quality of the man and of his mind, I think one may feel that what he did is likely to stand until it becomes obsolete with the advance of knowledge, as so often happens although the historical importance of the work remains.

In his work as a teacher of undergraduates and as a director of graduate students, Tennent was no less effective. The same thoroughness and determination to do his best characterized his teaching as it did his research. Tennent and I were gradrate students together at the Johns Hopkins and fellow members of Drew's Invertebrate staff at the Marine Laboratory. I well recall his first lecture to the Invertebrate class. He was so scared the chalk rattled against the board, but he did better than he thought and after that first summer the rest of us felt we must keep up with him. At the Hopkins he was not satisfied with his first year's seminar lectures. To be safe the next year, as he confided to me later, he went to the laboratory the evening before each lecture, turned on the lights in the empty seminar room and put himself through a dress rehearsal of his lecture to be given the next morning. It was this kind of determination and performance that characterized all his work. It had to be done as well as he could do it. I was told that E. A. Andrews went so far as to say at the time that Tennent was the best assistant he had ever had. Only those of us who were assistants to Andrews can fully appreciate what that meant as to quality of performance. Thus, Tennent had the instinct of workmanship at the beginning of his career.

Tennent always commanded the loyalty and admiration of graduate students to a marked degree. His great disappointment was that he did not train more students who were able to find places commensurate with their ability. Those in his confidence knew that he often longed for a position where he might have had more "disciples". He trained many women of ability, but for the most part, in this man's world, they could not find positions worthy of their competence. His summers at the Tortugas Laboratory gave him opportunities to extend the kind of contacts he might have had in larger measure throughout the year in some institutions. I have often heard of what a stimulus he was to the younger investigators at Tortugas summer after summer.

On the personal side, Tennent was always quiet and reserved, though in his later years as well as in his youth a delightful companion to those who knew him well. He may have seemed austere to those who knew him casually. Yet he had a keen sense of humor for all his quietness. I never knew a man whose sense of obligation to do what he thought just and right seemed to me stronger nor a man whom I would trust further. Thinking of him personally, one felt that here was a man to whom the abused and meaningless phrase "a gentleman and a scholar" might be applied with meaning.

To Mrs. Tennent and to his son, who as a

scientist follows in his father's footsteps, we extend our deepest sympathy.

W. C. Curtis

EDWARD BROWNING MEIGS

Edward Browning Meigs was born in Philadelphia September 10, 1879, the son of Arthur Vincent Meigs and Mary Roberts Browning. He belonged to an old and distinguished American family of South English ancestry, and was a direct descendant of Vincent Meigs who came to America and settled in New Haven, Connecticut, in 1644. His father, grandfather, and great grandfather were physicians; his great great grandfather, Josiah Meigs, was professor of mathematics and natural philosophy in Yale University in the 1790's. Scientific interests were strong in his ancestry. His father, a pediatrician, was interested in the chemical composition of milk and published papers on this subject; he also introduced a method of modifying cows' milk to make it suitable for infants. In Edward Meigs' memoir of his father he "finds it difficult to say whether more of my father's energy was devoted to the practice of medicine or to research".

Edward Meigs was the fourth physician in his family in the direct line, but his own interests were primarily scientific and he did not engage in practice. He graduated from Princeton University in 1900 and took his M.D. at the University of Pennsylvania in 1904. He was Assistant in Physiology in the same University during 1904-1906 and then spent a year abroad in study and research, working chiefly in Jena with the comparative physiologist Wilhelm Biedermann. He also spent some time in Cambridge University, chiefly in association with Walter Fletcher and Gowland Hopkins, whose work on the physiology and biochemistry of muscular contraction was of special interest to him. He was instructor of physiology in the Harvard Medical School during 1907-10. In 1910 he joined the Wistar Institute in Philadelphia in order to devote himself entirely to research. From 1915 until his death he was physiologist in the Bureau of Animal Industry of the U. S. Department of Agriculture.

He first attended the Marine Biological Laboratory in 1904, and was elected a member of the Corporation in 1905. During the four summers of 1912-1915 he served as instructor in the physiology course. He and his family have been summer residents of Woods Hole for a period of about thirty years, and his interest in the Laboratory has been constant.

In 1910 he married Margaret Wister of Philadelphia who with two sons and two daughters survives him. He died November 5, 1940, after a prolonged illness.

Edward Meigs' early investigations were in the field of general physiology, especially the physiology of muscular contraction. Later, after he went to Washington, his work had reference chiefly to the physiology of milk production and related topics; problems connected with administration and the organization of research in this field also engaged much of his attention.

His early studies on the comparative histology and biochemistry of smooth and striated muscle, both vertebrate and invertebrate, were varied and extensive. His photographs of striated muscle fibres under high magnification are among the best that we have. His belief that changes of tension in muscle were a result of reversible changes of hydration in the fibrils led him to experiment on the influence of variations of osmotic pressure, chemical conditions and temperature on the water content and correlated state of contraction of different types of muscle. At one time he was greatly interested in physical models of muscular contraction, especially McDougall's model, in which increase of volume of inflation resulted in shortening. His interest in the relation of inorganic salts to contraction (as shown e.g., in the potassium contraction of striated muscle) led him to make comparative analytical studies of the salt content of smooth and striated muscle, vertebrate and invertebrate. This work had an indirect but important bearing on his later work in the Department of Agriculture on mineral metabolism in its relation to milk production. He recognized that the selective action of the muscle cell in accumulating its highly special salt content had the same physiological basis as the selective separation of salts by the mammary gland in milk secretion. Problems of permeability also interested him in relation to both the properties of muscle and the processes of secretion; and in work on artificial membranes he showed that impregnation of collodion films with insoluble calcium and magnesium salts formed membranes approaching living plasma membranes in their semipermeability, a property which in the living cell also is dependent on calcium. He found, however, that the behavior of smooth muscle in anisotonic Ringer's solution and sugar solution differed from that of striated muscle and indicated the presence of a much less diffusion-proof surface layer. This difference in physical properties he correlated with other evidence of a fundamental difference in the mechanism of contraction in the two types of muscle.

During Edward Meigs' work of twenty-five years in the Department of Agriculture he and his associates made varied and important contributions to the physiology of milk production. Mineral metabolism and vitamin supply in relation to milk production received special attention, and the results of this work were published in a

long succession of special papers and reviews. He was also responsible for the general planning and direction of the work at the experimental farm at Beltsville, Maryland. Many problems of a highly practical kind also came up for consideration; for example the incidence of mastitis in the experimental herd led to an investigation of the pathology of this condition and effective methods for its control were developed, including modification of certain types of milking machine which were found to be largely responsible. The work of these years is too varied to summarize briefly; some of its practical results are seen in the progressive improvement during recent years in the general methods employed in the dairy industry.

Much of Edward Meigs' success in this work came from the thoroughness, objectivity and freedom from bias that were characteristic of his scientific activity and outlook. He was highly tenacious in his convictions once they were formed, but his conclusions were always based on a clear-sighted and critical consideration of evidence, in the collection of which he spared no pains. Although weakened in his later years by illness, he maintained his scientific interest and activity to the last. His personal interests other than scientific were remarkably wide; he was fond of nature and outdoor life, a great sailor, a man of imagination and culture, widely read, modest, loyal, highminded and devoted to his friends. His characteristic generosity was well shown in his gift to the Marine Biological Laboratory in 1936 of the bathing beach property, including the bath house, on the Buzzards Bay front adjoining his family summer cottage. The use of this beach by members of the Laboratory, as well as of the tennis courts which occupy part of his property is thus permanently assured.

R. S. Lillie

FURTHER IMPLICATIONS OF FLEXIBLE PROTEIN FRAMEWORKS

(Continued from page 177)

of intermediates, formed by condensations of suitable materials adsorbed on individual protein faces. This idea fits with the more detailed picture now emerging from enzyme studies, according to which there is a division of labor among the enzymes concerned in such syntheses, each enzyme catalyzing the condensation of certain building blocks only (Fruton, *Cold Spring Harbor Symposium*, 1941, forthcoming). Such a situation explains the existence of symmetric arrangements of R-groups such as are known to be present in insulin, for example It also fits with the existence of precursors such as Svedberg's prolactalbumin and several other protein intermediates, which curiously enough all have molecular weights in the neighborhood of a thousand, corresponding possibly to individual faces or other precisely defined fragments of 72-residue or 288-residue skeleton cage structures. Further, the protein frameworks offer, not only characteristic and specific individual protein faces as templates for the formation of these intermediates but, in the branch point protein units, provide nests where such platelet intermediates may find themselves in favorable juxtaposition for the final synthesis into complete cage molecules. In suggesting that such branch points in protein frameworks are the seat of protein synthesis, it may also be pointed out that the adsorption in such nests of a foreign molecule, e.g., any antigen, will in general provide templates of new specificities, leading to the formation of proteins of specificities complementary to those of the foreign molecule, e.g., antibodies. These pointers regarding protein intermediates and the mechanism by which a system imposes certain specificities on them could be utilized in the design of experiments.

(2) As our central and fundamental postulate it has been assumed that the proteins in such structure systems are in their native form. It follows that the interlinking of such units into frameworks also shows specificity. In connection with certain particular cases of one of the three types of such interlinkings, namely the riveting together of ionized groups by some divalent ions, certain suggestions regarding the function of such ions emerge.

The fact that young tomato plants grown in pyrex glass containers with nutrient solutions deficient in zinc give a measurable and reproducible response to the addition of one gamma of zinc to a plant (giving a zinc concentration in the culture solution of 5 parts per billion) may be taken as typical of many facts regarding the importance of minute concentrations of certain elements (Arnon and Stout, *Plant Physiology, 14,* 371, 1939). Copper in certain minute concentrations is of the first importance in many cases. As examples we may cite the well-known facts that the flagellate *Euglena* can live in a concentration of 10^{-8} M copper sulfate as long as food lasts, but dies in a few minutes when the concentration reaches 10^{-7} M (Seybold, *Biol. Zebtralb., 47,* 102, 1927); that *Amoeba proteus* cannot survive when the concentration of $CuCl_2$ rises as high as 2×10^{-8} M (Chalkley and Voegtlin, *U. S. Pub. Health Rep., 47,* 535, 1932); that lysis of certain red blood cells by glycerol can be inhibited by an amount of copper insufficient to cover more than one-tenth of one per cent of their surfaces; and so on. The fact that a fraction of a milligram of copper is just as necessary for the life cycle of a tomato plant as several hundred milligrams of potassium, for example, and the other facts cited, I would interpret as one more symptom of the

fundamental superspecificity of biologically active structure systems. For the specificity of individual native protein units, conjoined with specificity of interlinking to give the superspecificity of native protein frameworks implies the existence of certain definite complements of positions capable of accommodating the metallic ions. While we are not yet in possession of complete information as to all the atomic environments into which ions such as zinc, copper, calcium, etc. can fit there is already sufficient indication in structure analyses to indicate that each ion type has its own individual requirements, shown by the cation radius associated with each cation coordination number. It would seem then that the stoichiometry of native proteins and ions, especially in relation to what is already known about atomic environments appropriate to the various ionic species would afford a direct experimental approach to the part played by metallic ions in physiological situations in certain cases. Particularly significant in this connection are the many facts from various fields indicating the part played by calcium ions, which may most usefully be studied in the light of structure analyses. These include the increase of the agglutination of sperm in the presence of calcium recorded by Loeb in 1914; the capacity of a torn sea urchin egg to mend itself in the presence of $CuCl_2$ recorded by Chambers in 1924 (*Am. J. Physiol., 72,* 210, 1924); the studies on calcium caseinates by Philpot and Philpot in 1939 (*Proc. Roy. Soc. London, 127B,* 21, 1939) showing that particles of increasing weights are formed in solutions of increasing calcium concentrations. To my mind, all these suggest some specific interlinking of R-groups of native proteins, modelled perhaps on the atomic environments characteristic of the ions in $Ca(OH)_2$, etc., a suggestion which could be exploited in detail in carefully designed experiments. The fact that calcium is known to be present in the nuclear membrane indeed prompts the suggestion that calcium ions (or some similar ions) may play an important part in holding together the native proteins which no doubt constitute this and other biologically active membranes. In this case, a change in pH reducing the ionization of the acidic R-groups riveted together by the calcium ions, would necessarily lead to a collapse or dissolution of the membrane in dividing cells. The subsequent formation *de novo* of nuclear membranes in daughter cells would then require the accession of a definite though very small complement of calcium ions.

In conclusion, I would record my belief that the present-day picture of the native protein is pregnant with meaning for all structure problems in physiology, because it offers hints as to directions in which explanations of certain so far uninterpreted facts may be sought. I desire to put before those concerned with the design of experiments intended to throw light on the structure systems of living matter, the suggestion that it is the specific nature and behavior of individual native protein units which is the central theme both for the understanding of the synthesis of proteins and for the understanding of the part played by metallic ions in living matter. It has already been shown that by means of these simple ideas both the high water content and flexibility of living matter and the immense specificity and stoichiometric significance of living structure systems can be explained. Evidence is accumulating day-by-day that it is the intimate atomic structure of the protein molecule which dominates the scene. How such a situation could possibly be explained in terms of the curious hangover picture of polypeptide chains of immense length I leave to others to explain. How suggestively, on the other hand, the new picture of the native protein fits in and comes to the rescue will, I believe, become increasingly clear when the perfectly definite indications regarding its structure are made use of in the design of experiments on these essential constituents of living matter.

(This article is based upon a seminar report presented at the Marine Biological Laboratory on August 19.)

INVERTEBRATE CLASS NOTES

(Continued from page 184)

—five to four; the Inverts were victorious.

Right in the middle of the game, fourteen of the girls strolled quietly to the supply house. A well-dressed young man approached and was about to enter the supply house when the girls grabbed him by the arm and marched to the Eel Pond, while the hurdy-gurdy man played "Who's Afraid of the Big Bad Wolf?" His adversaries allowed him to remove his outer garments, after which he meekly plunged in. A new M.B.L. record had been established: the girls had thrown a man into the pond. The fellow had taken a former dip into the Pond ungraciously and had taken the matter to the authorities. A meeting was called at which time he was to identify those responsible. It was at this point the Invert Amazons stepped in. To commemorate this deed and to express their gratitude, the "boys that sweep the lab and the boys that bring the 'fish'" arranged a fitting rally on the Brick Lab steps. With the inimitable Jasper Trinkaus presiding, there was much banter, singing, and presentation of flowers.

A strange figure looked in on the unusually hilarious group Tuesday evening, remarked: "My God! this course isn't what it was when I took it forty-five years ago!"

Saturday, August 30, is the last day of the course. We are especially grateful to the staff and crew; this has been an unforgetable experience. —*Louise Gross and Bill Batchelor*